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DR. WILLIAM H. PARK, *President.*

RUPTURE OF THE AORTA DUE TO CARCINOMA OF THE ESOPHAGUS.

GEORGE P. BIGGS, M.D.

This specimen was removed from a female aged forty-four. For three months prior to admission to the Hospital, she was unable to swallow solid food and only small quantities of liquid. Previous to that for a year,

there had been nausea and a great deal of intestinal trouble, without any vomiting. She had lost slightly in weight during the previous six months. Examination by the bougie showed fairly marked stricture of the esophagus, twelve inches from the line of the teeth. Shortly after admission gastrostomy was performed and a bougie ½inch in diameter passed through the stricture from below, obstruction being first encountered 3½ inches from the cardiac orifice.

There was a little improvement in the power of deglutition following this, but about two months later it was again noted that there was inability to swallow anything but small quantities of liquid. The fistula into the stomach was kept open and nourishment was administered in that manner.

Three months after operation during one entire day there were frequently small hemorrhages from the esophagus. The dressings about the abdominal wound became thoroughly saturated with blood and that evening, following a very large hemorrhage, death resulted. On postmortem examination the stomach was found greatly distended with blood. A smaller quantity of blood was found in the small intestines and in the bronchi. The growth in the esophagus was rather extensive in its involvement beginning on a level with the bifurcation of the trachea, and involving the entire circumference for a distance of eight centimeters below that point. There was apparently no marked stricture at this time, but a very extensive ulceration of the entire involved area.

A little below the center of this ulcerated area, was found a small opening leading directly into the aorta, communicating with its interior by an opening about two millimeters in diameter. The site of this communication as viewed from the aorta was further marked by a

thrombus one and one-half centimeters in its greatest diameter with a very small base of attachment near the opening.

The tumor is rather of the scirrhus type of carcinoma. Section was taken from a point not very near the perforation in order not to destroy the specimen. There was but very little inflammation, and no necrosis except very superficially.

I have not seen such a specimen of complete invasion of an artery of very large size by any new growth, though occasionally they are found in large veins. In no other case have I seen evidence that death was due to extensive hemorrhage following erosion of a large vessel by a new growth. Without previous knowledge of this case, the final symptons would have suggested rupture of a thoracic aneurism.

The other case I wish to speak of is a pistol shot wound of the skull, without any injury to the dura but with laceration of the pia resulting in death. The case was that of a man fifty years old, who shot himself in the upper portion of the right temporal region. The size of the ball I was unable to obtain. It had been removed at operation. There was a depressed fracture at the point of contact of the bullet and the man was comatose when admitted to the Hospital. The bullet was found just beneath the scalp, a short distance from the point of entrance. All fractured bone was removed and the dura was found to be uninjured apparently, though no distinct pulsation was noted. The conditions did not seem to warrant further exploration. There was no material improvement after the operation and the man died a few hours later. At the examination of the wound post-mortem, the dura was found entirely uninjured so far as

any gross inspection could show. It was very tense, owing to a very large sub-dural hemorrhage causing marked pressure, and apparently being the immediate cause of death. In the pia, just beneath the central portion of the fractured area in the skull, was a linear tear 1½ centimeters in length crossing one of the main branches of the middle cerebral artery. The convolution beneath this point of laceration of the pia had its normal contour and was not damaged so far as examination of the exterior could reveal, but upon incision a large number of small petechial hemorrhages were found in the gray matter. The case was to the surgeons, evidently, rather unusual, as they did not think it necessary to go further after finding the dura uninjured. The blow of the bullet made a degree of pressure sufficient to tear the pia without producing any visible damage to the dura.

The third specimen is a fresh one which is of slight interest as a very unusual thrombosis of the left ventricle. The history of the case is very long. Many points of interest from a clinical standpoint, I will not refer to, but simply present the specimen as a rather unusal one in the line of cardiac thrombi. It was from a male aged 42, who first came under observation during an attack of typical uremic coma which developed while at work. During the 69 days at the hospital, he was in a more or less comatose state. Death was expected almost any day during the entire time. The urine and general symptoms were such as to make it very clear that it was a case of contracted kidney. The autopsy revealed lesions which would make a good nucleus for a pathological museum in the way of changes more or less closely related to kidney lesions, including extensive thrombi. One of the latter was in the apex of the left ventricle. It was a pear

shaped thrombus attached by its small end to the very apex of the ventricle at two points, one of them a mere thread; the other an area of about one centimeter by one-fourth centimeter, the large end of the thrombus projecting perfectly free within the ventricle. It was of the firm, white variety and measured about 4½ cm. in length, and 2¼ cm. in greatest diameter. Attached to the anterior wall of the left ventricle above the apex was another small thrombus 1 cm. in diameter, ¼ cm. in thickness, which had been distinctly flattened by the larger one. One kidney, weighing 100 grammes, was exhibited with the heart.

SOME INTERESTING CASES OF ENDOCARDITIS.

E. LIBMAN, M.D.

Case I. Staphylococcemia in a Case of Chronic Endocarditis.

The patient from whom the specimen was obtained was a man aged 36 years who was admitted into the Mt. Sinai Hospital in Dr. Brill's service, with symptoms of aortic insufficiency and mitral stenosis. For about ten days he had very slight temperatures; on the eleventh day the temperature rose to 106° F. and he developed petechiae on the surface of the body and on the mucous membranes. A blood culture made the following day showed the presence of the staphylococcus aureus in large numbers; two days later the blood showed the same organism. The temperature continued quite high and the man died in about twelve days after the onset of the acute symptoms.

The post-mortem examination revealed an aortic insufficiency and a mitral stenosis, as had been suspected

during life. There were no acute lesions of the valves except for a very minute vegetations on the edge of the aortic flaps. On the endocardium, just below the posterior aortic flaps there was a deposit of fibrinous material measuring 1½ by 2 cm. in width and length and about 3 mm. in thickness. There were innumerable miliary abscesses in the kidneys and heart muscle and infarctions in the kidneys and spleen; areas of acute interstitial myocarditis were also found. The cultures from the heart blood post-mortem revealed staphyloccus aureus pure and the same organism was isolated from a number of metastatic abscesses. The fibrinous-looking area on the endocardium, on microscopic examination, proved to be made up almost entirely of staphylococci. The metastic abcesses showed staphylococci in spreads.

The point of interest about this case was that, although the case was one in which a clinical diagnosis could easily have been made of ulcerative endocarditis involving the already diseased flaps, these were found practically negative at post-mortem. The case demonstrated very well the importance of being careful before making a diagnosis of ulcerative endocarditis even in the presence of distinct physical signs of disease of the valves.

Case II. Fatal Rupture of a Pulmonary Adhesion in a Case of Mitral and Aortic Stenosis.

This heart came from a woman 36 years of age. Several days before admission into the Hospital (Dr. Brill's service) she was taken sick suddenly with pain in the right chest and went into collapse. On admission into the hospital she was almost pulseless and very pale. The clinical diagnosis was mitral stenosis, thrombosis in the auricles and effusion in the right chest. On aspirat-

ing the chest, fluid blood was obtained. It was suspected that the patient was suffering from a ruptured infarction of the lung.

The post-mortem examination revealed the following: The aortic orifice was almost completely stenosed; the mitral orifice barely admitted the end of a lead pencil; there were apparent thrombi in the auricles; the right pleural cavity contained a large amount of blood. On the inner surface of the sixth rib there was found a firmly attached small strip of lung tissue. On careful examination it was found that there had been a firm adhesion between the lower part of the right upper lobe of the lung and the chest wall and that the hemorrhage into the pleural cavity had come from a rupture of this adhesion. In the part of the adhesion attached to the lung was found a torn varicose branch of one of the pulmonary veins, so that although the clinical diagnosis of thrombosis in the auricles was proven to be correct, the hemorrhage into the pleura had nothing to do with the existence of these lesions.

Cases III and IV. Acute Endocarditis of the Aortic Valve with Perforation into the Right Auricle.

These two hearts illustrate a special point. The first case was that of a girl aged 18 years, who became profoundly septic after a slight injury to the right wrist joint, resulting in osteomyelitis of the ulna. There were numerous chills followed by very high temperatures and a marked progressive anemia. No petechiæ were found anywhere. Within the course of two weeks there was developed a distinct aortic insufficiency. The first blood culture showed no organisms. In the second culture 25 c.c. of blood were used and the staphylococcus aureus was found pure. It is interesting to note that the or-

ganisms were so few in the blood that they were found in only one flask of bouillon (four plates of glucose-agar had been used and three flasks of glucose-bouillon) and in this flask they were found only after three days. I hesitated in agreeing with the clinical diagnosis of ulcerative endocarditis until the organisms had been found in the blood, because in cases of acute ulcerative endocarditis I have always found organisms in the blood.

On post-mortem examination there was found vegetative endocarditis of both posterior flaps of the aortic valve; the infectious process had extended through the wall of the heart and there was a perforation into the right auricle just between two of the flaps of the tricuspid valve; there were firm vegetations around the place of perforation. The extension into the auricle had started from the posterior inferior part of the right sinus of Valsalva. The heart blood post-mortem was found to contain a pure culture of staphylococcus aureus and staphylococci were found in the spreads made from the vegetations.

This fourth heart was obtained from a woman aged 32 years whose symptoms were those typical of ulcerative endocarditis. There was present an aortic insufficiency and mitral stenosis. There is a marked vegetative endocarditis involving the aortic valve, and a perforation into the right auricle in the same position as in the third heart. In this case the streptococcus was cultivated from the blood during life and post-mortem. These two cases should properly be classed as endomyocarditis, for the myocardium is as much involved as the valve. They illustrate very well a point recently made by Krauss. In a recent article in the *Berl. Klin. Wochenschrift* he points out that the right sinus of Valsalva is a *locus minoris resistentiæ*. Aneurysms and perforations are

particularly apt to occur in this sinus. Krauss points out that if the aneurysmatic process involves the upper anterior part of the sinus, rupture is apt to occur into the right ventricle just at or below the origin of the pulmonary artery, but if the aneurysmatic process involves the posterior inferior part of the sinus, the perforation is apt to occur into the right ventricle just beneath the tricuspid valve in the situation of the so-called pars membranacea of the septum, or into the right auricle between two of the flaps of the tricuspid valve.

I have brought with me a heart which I showed to the Society two years ago, which shows a compression of the conus of the pulmonary artery due to the inflammatory swelling in the septum secondary to an ulcerative endocarditis of the aortic flaps and the sinuses of Valsalva. In this case the most marked ulceration was found in the upper anterior part of the right sinus of Valsalva.

Discussion.

Dr. Ewing said that he had been very much interested and surprised to find in such a considerable proportion of the cases staphylococci appearing as causes of the lesions. In his experience in examining blood in cases of bacterial endocarditis, he had found the streptococcus and never the staphylococcus. He asked whether the cultures were made before or after death. If before death, how long before, and if they had not been obtained before death—say twenty-four hours—what relation Dr. Libman regarded that they must have with the lesion itself, and how they were to be separated from secondary infections.

Dr. C. W. Field said that at the Presbyterian Hospital they had found that the blood cultures taken by the House Staff showed contaminations in about fifty per

cent of the tubes which showed any growth. In spite of careful asepsis, contaminations will occur. Dr. Tuttle threw out any case in which he found staphylococci. In many cases some staphylococci from the skin were found. Dr. Field thought that in the last case in which one staphylococcus was found in twenty-five cubic centimeters, the organism could have been obtained from the skin.

Dr. Libman said that in his opinion there was no question about bacteriologic findings in the cases which he had described. He did not consider it diffiicult to avoid contamination of the cultures if one had had experience. The patient from whose blood he had made two cultures was profoundly septic at the time of admission to the hospital, so that the question of infection from taking a culture did not need to be considered. The staphylococcus pyogenes aureus was an organism concerning the presence or absence of which there could be no doubt if all the staphylococci that were found in the cultures were carefully examined. He wished to draw attention to the fact that one must be careful in reporting the presence of streptococci in the blood, for the reason that there are contaminating organisms which grow on media without sugar like ordinary streptococci. On sugar media they could be readily distinguished as they formed large mucoid colonies. The two organisms of this group which he had seen were from Gram negative.

Dr. F. C. Wood said that he felt that he must defend the pathological internes at St. Luke's, for in comparison to the results of the staff at the Presbyterian, contaminations were exceedingly rare with them. Their trouble was that they did not get any growth in a considerable number of cases from which one naturally expected to obtain some organisms. The cultures remained

perfectly sterile and rarely showed contaminations. The system which was in use there was to plant a large series of broth tubes or flasks and then plate from these later.

Dr. W. H. Park said he thought one thing should be remembered: that where cultures were made continuously by experienced observers like Dr. Libman the results were more reliable than when made by hospital internes. He had had considerable experience in making blood cultures in malignant endocarditis. In nearly all cases the organisms found were streptococci. He had been afraid to consider that staphylococci came from the blood if they appeared in one flask only, when several were inoculated, for fear that the growth might have been due to skin infection.

Dr. G. P. Biggs said that in septic cases at the New York Hospital they had had no particular trouble in getting positive results from blood cultures, but had found the streptococci much more frequently than staphylococci. He desired to commend the work of the House Staff in making these cultures. Thorough precautions were taken and contaminations were very few.

Dr. Libman said he believed that he had stated in the description of the first case that the blood culture was made twelve days ante-mortem and was repeated ten days ante-mortem, and that the same organisms which were found at that time were found in the heart blood post-mortem and in spreads made from the surface of the endocardium and from the metastases. A specimen which he had to show under the microscope showed very beautifully that the fibrinous area on the heart walls consisted entirely of staphylococci. In the last case which he had reported the streptococci were found in the blood one month ante-mortem and twice on later occasions. He had made blood cultures in over three hundred and

fifty cases of septic conditions (this did not include cases of typhoid fever or pneumonia) and in all cases in which he had found organisms present in the in the blood he had found the same organism present in the primary focus in the case, or in the metastases, or in both; often the same organism was found in the urine, so that there was no doubt about the validity of any of the results in the cases reported. Dr. Libman said that he had frequently found the staphylococcus aureus in the circulating blood; there was no reason why it should not be found as well as the streptococcus. In only one case had he found the staphylococcus albus in the blood. This was in a case of endocarditis which he had showed to the Society two years before. In that case numerous colonies had been obtained and the spreads which had been made from the inflammatory swelling in the septum of the heart post-mortem showed innumerable staphylococci. The culture from the swelling showed the staphylococcus albus. He believed that at that time he might very well have been dealing with the staphylococcus aureus because he had not then made it a point to test with sufficient care all organisms which looked like the staphylococcus albus. In the last four years he had not come across a case in which he could ascribe a systemic sepsis to the staphylococcus albus. The finding of the staphylococcus aureus, however, if blood cultures were made properly, was of just as much significance as finding a streptococcus. In a number of cases they had found that the organisms often did not persist for a long time in the blood, they settled in various parts of the body and formed metastases. In some cases of endocarditis, however, they had found streptococci in the blood repeatedly. In three cases they had found them frequently during the course of several months; two of these cases had left the hospi-

tal with slight temperatures. One had died and examination showed endocarditis of the left auricle: streptococci were found in the heart blood.

Dr. Field asked who took the blood cultures and whether they were taken by the members of the House Staff.

Dr. Libman said they were nearly all taken by himself. He had had only two members of the House Staff take them in the last three years. They gained experience for a month or two on cases from which no organisms were expected. After they had obtained sterile results in such cases they were allowed to take blood cultures which Dr. Libman was willing to count in his statistics. Only those men who had already done some work in bacteriology were permitted to make the cultures. If a man could not obtain cultures without contaminations Dr. Libman said he would not be willing to have him take one in a case which he was anxious to include in his studies. The blood cultures in the cases which he had reported had all been made by himself.

In reply to Dr. Park, Dr. Libman said he was rather surprised that there should be any doubt as to the finding of the staphylococcus aureus in the cases which he had reported. As he had stated before he had no difficulty in finding staphylococci and in confirming his results. He had not found the staphylococcus albus on his plates. He had occasionally got colonies of ordinary moulds or organisms which could at once be recognized as being non-pathogenic organisms, but these were generally found after the flasks had been examined once or twice. He felt so sure of a systemic infection by the staphylococcus aureus when he obtained it in its blood cultures that he had recently been willing to state that there was a systemic infection by the staphylococcus aureus in a case

in which he had found only one colony on the plates where five c.c. of blood had been used.* This blood was obtained from a man who presented signs of severe sepsis which developed shortly after he had had a slight infection on his foot. The day after he had reported that the sepsis was due to the staphylococcus aureus, Dr. Libman said, several intramuscular metastases were opened and the staphylococcus aureus was found in spreads and cultures. The man was operated upon several days later for a large metastatic abscess of the right kidney and the same organism was found. Dr. Libman thought this case demonstrated very well the fact that staphylococcus aureus could be found in blood cultures and that the finding could be relied upon.

DIFFERENTIATION OF MONKEY AND HUMAN BLOOD BY THE SERUM TEST.

JAMES EWING, M.D.

At a previous meeting of the Pathological Society, the speaker presented the results of some experiments which tended to show that, to get any specific result in the differentiation of animal bloods by the precipitation of albumens by specific anti-sera, one must consider not only the dilution of the blood to be tested but even more carefully the dilution of the test-serum. In those experiments it was shown that in dealing with a very active beef-rabbit serum, one could obtain precipitates with quite a variety of bloods other than beef, if the test-serum was not diluted more than 1 to 5. When, however, the beef-rabbit serum was diluted 1 to 30, only beef and goat

* In correctly stated at the meeting as 25 c.c. *(E. L.)*

blood reacted while at the dilution of 1 to 50 goat blood failed to react, while the beef blood still gave a prompt precipitate. The dilution of the blood to be tested, if under 1 to 500, made comparatively little difference. These results were very similar to those previously obtained with the same method by Kister and Wolff (*Zeit. f. Hyg.*, Bd. 41) but quite different in some minor respects.

I have since then attempted to see to what extent monkey blood of various accessible types reacts to humanized sera. For this purpose I obtained blood serum from four monkeys representing three different breeds and have tested these sera with four different strains of humanized sera. The monkeys tested were two Java, one rhoesus, and one baboon, whose full generic names I cannot at present give. The test-sera used were two rabbit sera, called No. 1, and No. 2, prepared by Dr. Strauss in the Cornell Laboratory, one rabbit serum, called No. 3, prepared by Dr. Payne in the Laboratory of the Department of Health, and one chicken serum, called No. 4, prepared by myself. I am greatly indebted to these gentlemen for the supply of these sera.

The strength of the sera may be indicated in various ways, perhaps best by the highest dilution in which they yielded a flocculent precipitate in human blood, diluted 1 to 50 or 1 to 100, within three hours or less.

The following sera gave flocculent precipitates in one month old human serum.

Test serum	Dilution of tested serum	Dilution of test-serum	Time
No. 1	1–100	1–50	30 min.
No. 2	1–100	1–50	3 hours
No. 3	1–100	1–200	1 hours
No. 4	1–100	1–50	1½ hours

Serum No. 3 (Dr. Payne's) was extremely active, giving an instantaneous turbidity when added in a dilution of 1–100 to human serum diluted 1–1000. Serum No. 4 (Chicken serum) was distinctly more active than No. 1 or No. 2.

The result with the use of these sera on monkey serum was briefly as follows:

1. *Baboon Serum.* In all dilutions of test sera Nos. 1, 2, and 3, up to and including 1–30, the baboon serum gave flocculent precipitates or turbidities within three hours, which were indistinguishable from many reactions encountered in testing known human blood. This was specially true with serum No. 3 which precipitated baboon serum about as actively as human, up to 1–20, and gave a very slight flocculent precipitate in three hours, when added in dilution of 1–40 to baboon serum diluted 1–200. These precipitates were, however, much less marked than in the corresponding human controls. Even with the active serum No. 3 baboon blood in any dilution failed to yield a flocculent precipitate in three hours when the test serum was diluted 1 to 50. The reactions with sera Nos. 1 and 2 were less marked, and the pronounced turbidities occurring usually, failed to become flocculent in three hours.

2. *Rhoesus Serum.* The reactions with this serum were almost identical with those obtained with the baboon. Sera Nos. 1 and 2 failed to give flocculent precipitates in any but the earlier dilutions (1–10, 1–20) within 3 hours, although causing prompt and distinct turbidities. Serum No. 3, however, gave abundant flocculent precipitates in three hours or less, up to dilutions of the test-serum 1–40, beyond which the turbidities failed to become flocculent in three hours, plus. The dilution

of the monkey serum if not beyond 1–200 made very little difference in the results.

3. *Java Serum.* This serum reacted more like human blood than did either of the others. With serum No. 1 it gave distinct turbidities up to a dilution of test-serum 1–40, but the turbidities failed to become flocculent. With serum No. 3, a flocculent precipitate was obtained in 90 minutes, tested serum 1–100, test-serum 1-80. With test-serum 1–100 distinct turbidities occurred promptly but failed to become flocculent. With test-serum 1–200 all trace of reaction failed although in the human control a flocculent precipitate occured in one hour.

From the results with these humanized rabbit sera it appears to be possible to distinguish from human blood, that of certain monkeys by using high dilutions of test sera, the strength of which has been accurately calibrated. The strong sera No. 3 gave the most satisfactory results, since in high dilutions (1–100, 1–200) it failed to react with monkey blood, while still actively precipitating albumens of human blood. With all lower dilutions, however, this serum gave turbidities or precipitates in both bloods, which would not permit the separation of the monkey from the human.

The suggestion arose that an animal more distantly removed from man, might yield a more selective serum. An ordinary hen was therefore treated with 6 intraperitoneal injections of 10 c.c. of defibrinated placental blood, at intervals of 2 to 3 days. Last injection February 5th, serum drawn February 9th. This animal did not react to these injections by any visible symptoms. Its serum, previously inert, was found to precipitate the

above specimen of old human serum rather actively, as shown in the table.

When tested on Java and Rhoesus serum it failed to give flocculent precipitate in 18 hours in any dilution and gave only the very faintest turbidities in the earliest dilution, 1–10, beyond which the monkey serum remained clear till developing bacterial growth.

This single experiment does not, of course, demonstrate that all humanized chicken sera will prove so selective, and I have not had time to repeat the experiments with other chickens. In any event it would seem that the chicken may be recommended for this class of experiments. It is easily handled, is cheap, receives injections without apparent reaction, yields blood freely from superficial veins in the wing, stands hemorrhage well, and is apparently immune to most ordinary infections.

I conclude from these experiments that monkey blood can be separated from human blood under suitable conditions. One must know accurately the strength of the test-serum used. The stronger the serum, the greater is the danger of confusing monkey with human blood if slight dilutions are used, while with high dilutions the strong sera yield flocculent preciptates long after monkey blood has ceased to react. Very strong serum must be diluted at least 1–100, better 1–200, and confidence placed only in flocculent precipitates occurring within three hours or with a certain promptness.

Humanized chicken serum seems to be extremely selective for human blood and shows less antagonism than humanized rabbit serum against monkey blood.

Discussion.

Dr. W. H. Park said he did not understand the

necessity of using a dilution of one part of albumin or serum to one hundred of normal salt solution in order to get a complete reaction. If a series of tubes were arranged containing .1%, 1%, 5%, and 10% of serum and the solution of precipitin was gradually added there was found first a precipitate in the .1% and then in the 1% solution. If a stop was made there little change was found in the 5% and 10% solutions. If, however, as much more precipitin was added as there was substance to be precipitated as complete a reaction was obtained in the five per cent. or ten per cent. solutions as in the one per cent. with five or ten times as much precipitate. An amount of precipitin much too little to influence all of the albumin in solution did not completely act on a small portion but acted a little on all so that little effect was manifest.

Dr. Harlow Brooks said that he thought Dr. Ewing's work was of the greatest importance since it promised to throw some light on the ever interesting question of evolution and might assist veryma terially in determining more accurately the position which the various animals really occupied in their relation to man. He did object, however, to the text of Dr. Ewing's conclusions since he stated that the blood of monkeys could be definitely distinguished from the human and based his conclusions on the results of his experiments which had been conducted with the lower members of the monkey family only. No one doubted but that there was a very wide space intervening between man and these lower types of the primates. Huxley had showed long ago that fully as wide a space existed anatomically and mentally between the lower primates and the apes as between the apes and man. Dr. Ewing might about as well have compared

the blood of man with that of the dog family as with that of the baboon. The crucial test was to make such experiments with the blood of the highest primates, and only after Dr. Ewing had shown that a marked difference existed between the blood of these animals and man would he be justified in making so sweeping a conclusion as he had given. Dr. Brooks ventured to predict that Dr. Ewing would find an even wider variance between the sera of the orang and the lemurs than between that of man and the orang. The question could not be considered as settled until such determinations had been made.

Dr. Charles Norris thought that Dr. Brooks' point was well taken, for Nuttall and Grünbaum had not been able to distinguish the blood of the higher monkeys, the primates, from human blood by the precipitin test. With the blood of other species of monkeys differences had been noted.

Dr. Dinkelspiel said he thought the results obtained by Dr. Ewing spoke for themselves. By calibrating the dilutions he had derived from them a method of separation of monkey blood from human blood, a consideration which was not taken up at Cambridge, They had there used pure serum and mixed it with the original blood diluted one to one hundred. He thought this was where they had made a mistake as the sera were too strong. They had used the blood of the ox, sheep and deer, and such homologous bloods as that of the apes and human blood. He thought the line on which Dr. Ewing was working was in the right direction.

Dr. Wood asked Dr. Ewing if he had noticed any variations in the reactions between his standard sera and human blood from different healthy individuals. In some experiments which he had made during the past summer

in testing immune rabbits blood with a long series of specimens of blood from normal persons and with accurate dilutions, he had noticed a very considerable difference in the amount of precipitate produced and also in the time of the appearance of the reaction.

Dr. Ewing, in answer to Dr. Park's question as to why the chicken gave such a selective serum, that it would appear reasonable to suppose that this selective quality had resulted from the failure of the injections to excite any great disturbance of the viscera. Rabbits showed considerable disturbance, pain, restlessness, increased micturition, etc., after injections of beef and human blood, and these disturbances were probably accompanied by the production of various peculiar products of metabolism which might have interfered with the selective action of blood precipitins. The chicken showed no disturbance whatever that he could detect and it developed a very specific serum. Or the result might have depended upon the very distant removal of the chicken from man. In answer to Dr. Wood's question, he said that he had not found any great difference in the reaction given by various specimens of fresh human blood; but old specimens had seemed to react less promptly. Greater differences arose from the age of the test serum. Dr. Norris had referred to the well-known work of Nuttall and Dinkelspiel, and of Grünbaum, who had found great similarity in serum reactions between monkey and human blood. He did not remember from any of their publications that they had used carefully calibrated sera in increasing dilutions after the method first employed by Kister and Wolff, and on which alone the results of the present work depended. Doubtless he had not seen some articles which others had encountered, but he knew

of no previous attempt to distinguish monkey from human blood by this particular method. Dr. Brooks had pointed out very opportunely the limitations of the present set of experiments, and had shown that they could apply with certainty only to the lower monkeys. He had realized this limitation and did not claim to have determined as a fact that any monkey blood whatever could be separated from human blood by this method, but only that of the baboon, rhoesus, and Java monkeys. He had not been able to secure any blood from the higher anthropoid monkeys, Perhaps Dr. Brooks could furnish such specimens through his official connection with the Zoological Garden. There was, however, a great difference in the comparison of lower monkey blood with human, and dog blood with human. Dog blood could hardly be confused in the test with human blood while monkey blood was separated with difficulty and only by high dilutions of serum. The lower monkeys might appear to zoologists to be as far away from the anthropoid monkeys as was the dog from the lower monkeys, but there was not room enough in the scale of serum reactions for an equal difference to exist between the bloods of high and low monkeys. Hence, he felt rather confident that the blood of the anthropoid apes would be found to fall in the general class with the lower monkeys and would be found separable from human blood by calibrated serum. He would anticipate no greater difficulty in this than was encountered in separating beef, and sheep by beef-rabbit serum, as the beef and sheep were probably as closely related as were the man and ape.

THE RESULTS OF INTRAVENOUS INJECTIONS OF DILUTE FORMALIN SOLUTION IN SEPTICAEMIC RABBITS.

WM. H. PARK, M.D., AND WM. A. PAYNE, M.D.

This work was undertaken at the Research Laboratory, Department of Health, after the announcement of a cure of septicæmia due to the streptococcus had appeared in the public press. In all of our experiments, first the culture, then afterwards the formalin solution were injected intravenously; new rabbits were employed; and controls were always made. In order to roughly determine the effect of intravenous injections of formalin in rabbits, we injected a number of rabbits with varying dilutions and amounts. We found one c.c. of pure formalin to be immediately fatal; five c.c. of a 1–10 solution to produce alarming convulsions, later anæmia, paralysis and death; ten c.c. of a 1–100 dilution to produce slight temporary disturbances with following anæmia. Ten c.c., twenty c.c., thirty c.c., and fifty c.c. of a 1–5000 solution produced no apparent immediate disturbance, but one hundred c.c. caused abortion in a pregnant rabbit a few hours afterward. This may have been due, not to the formaldehyde, but to the large amount of fluid injected. One rabbit died during the inoculation of fifty c.c. of this same dilution. As far as immediate danger is concerned, ten c.c. of a 1–100 dilution seems to be within the bounds of safety for a full grown rabbit. Ten c.c. of a 1–200 dilution given a young rabbit was followed by a pronounced œdema of both ears. Rabbits receiving large amounts of formalin showed after two to three weeks emaciation and pronounced anæmia.

The quantity of formalin which could safely be given having been determined we injected a number of rabbits

intravenously with from one to ten c.c. of an ascitic broth culture of two strains of slightly virulent streptococci recently obtained from human blood, an amount sufficient to kill probably in from one to four days. Of each set of rabbits a certain proportion after inoculation received intravenous injection of formalin solution. About half of the rabbits injected are presented on the following table. The results in the others were similar. One rabbit which received one hundred c.c. of a 1–50000 solution of formalin, twenty-four hours before the streptococcus lived considerably longer than the controls.

TABLE I.

Showing results of intravenous injection of dilute formalin solution in rabbits, one to two hours after infection with streptococci from cultures.

A and B.

Rabbit Exper. Number	Amount of Culture injected.	Amount of Formalin injected.	Duration of life.	Smears and Cultures at death showed in blood.
1	10 c.c.	50 c.c., 1–5000 solution ..	18 hours	Abundant streptococci.
1	10 c.c.	Control; no formalin....	22 hours	" " "
1	10 c.c.	" " "	24 hours	" " "
2	10 c.c.	10 c.c., 1–200 solution ...	6 days	" " "
2	10 c.c.	10 c.c., normal salt sol...	15 days	" " "
2	10 c.c.	Control.....	10 days	" " "

The slight virulence of the streptococci in the above series of experiments hampered us in deducting conclusions as to the amount of protection, if any, afforded; but we used these rabbits to demonstrate that after even large doses of formalin the streptococci can still increase in the blood and cause death through septicæmia. All the rabbits receiving formalin after the streptococcus died before those receiving the streptococcus alone. In the other table we show another series of rabbits which were in-

jected with a virulent culture of pneumococcus sent to us by Dr. Wadsworth. Here as before with the streptococci, the formalin failed to give the least protection Table II. gives the results in eight rabbits, four of which received the formalin dilutions after the pneumococci.

TABLE II.

Showing results of intravenous injections of formalin solution in rabbits within one hour after the injection of a surely fatal dose of a virulent pneumococcus.

A got pneumococci alone; *B* got pneumocci and later formalin solution.

Amount of culture used.	Date.	Amt. and dilution of formalin used.	Time of formalin injections.	Duration of life.	
No. 23 A 1 c.c.	Jan. 27	None		1 day	Smears and cult.
" 24 B 1 "	" 27	5 c.c., 1–100	1 hour after	1 day	at death showed
" 25 A 5 "	" 28	None		3 days	in blood abundant
" 26 B 5 "	" 28	5 c.c., salt sol. 5 c.c., 1–100	Immediate	2 days	pneumococci,
" 27 A 5 "	" 31	None		4 days	" "
" 28 B 5 "	" 31	15 c.c., 1–100	1 hour after	2 days	
" 29 A 1 "	Feb. 6	None		3 days	" "
" 30 B 1 "	" 6	10 c.c., 1–1000	30 mins. after	2 days	

From the tables it is seen that the injections of formalin were given in some cases equal to and in others far beyond the comparable dose advised by Barrows in man, and yet these rabbits died uniformly before the controls. Although these experiments are limited in number yet they are so uniform in their outcome, that it seems fair to assume that non-lethal formalin injections cannot stop a septicæmia already started in healthy animals. The results of Fortesque and Bricksdale, published just as we were finishing these tests, agree in every point with ours. They used anthrax bacilli and streptococci.

During these experiments streptococci have been

obtained from the blood of four cases of streptococcus septicæmia in man. Each of these cases received two or more intravenous injections of from five hundred to seven hundred and fifty c.c. of a 1–5000 solution of formalin. All four died. Their deaths took place in from five days to three weeks after the injection of formalin solution. (*Lancet*, January 10, 1903.)

The experiments reported here and elsewhere, and the fatal outcome of septicæmia in man after diluted formalin injections should cause us to be guarded in using formalin, and make it necessary to weigh each case injected not only to determine when improvement ensues whether recovery has been promoted, but also when death occurs as to whether harm has been done. An intravenous injection of water plus sodium chloride may prove better than water plus formalin.

OBSERVATIONS ON THE BLOOD OF RABBITS INTO WHICH FORMALIN HAD BEEN INTRAVENOUSLY INJECTED.

L. B. GOLDHORN, M.D.

At the request of Dr. Park, some observations were made on the blood of the rabbits used for formalin injections, as it was thought possible that changes might be detectable in the general morphology or in the percentages of the leucocytes. It was found that no changes in the granulæ of leucocytes took place, and that the relative percentage of the different forms remained

rather constant; but an anæmia developed invariably after the formalin injections. The degree of anæmia depended upon the amount and strength of the solution injected. It evidenced itself by an increase in the polychromatophilic disks, varying degrees of poikilocytosis, and the presence of blasts in some cases. In a rabbit which received an injection of ten c.c. of formalin, 1–5000, on January 16, 1903, and five c.c. of formalin, 1–10, on January 20, 1903, the anæmia was very extensive and a polynuclear leucocytosis appeared. The lymphocytes were most interesting, inasmuch as they showed nuclear division phenomena. This particular rabbit was kept alive under observation until February 22, 1903, when it died. It exhibited peculiar symptoms; i. e., the reflexes were heightened, and there was loss of co-ordination. Temperature fluctuated between 39.5° and 40° C., and there was no nephritis.

The rabbit which showed the peculiar high percentage of lymphocytes was autopsied and an extensive coccidosis of the liver was found besides a considerable increase in peritoneal fluid.

The following tables will in a general way show the observations made:—

Date	Polynuclears	Lymphocytes	
Jan. 31, 1903 Injected 5 c.c., 1–100 Formalin. 30 mins. later	18.5%	75.5%	
Jan. 31, 1903	18.5%	73.5%	
Feb. 1, "	13.5%	84 %	
" 5, "	18.4%	76.4%	
" 7, "	19.2%	74.8%	Beginning anæmia
" 11, "	16.3%	77.8%	Anæmia

Autopsy: Coccidiosis of liver. Ascites.

Date	Polynuclears	Lymphocytes	
Jan. 31, 1903	43.6%	48.8%	Inj. of pneumococcus 1 hour before.
Injected 15 c.c., 1–100 Formalin.			
Jan. 31, 1903	47.3%	48.7%	
Feb. 1, 1903	50.2%	44.5%	Nucleated red cells present
Death.			

Date	Polynuclears	Lymphocytes	
Jan. 16, 1903			
10 c.c. Formalin, 1–5000			
Jan. 20, 1903			
5 c.c. Formalin, 1–10			
Jan. 31, 1903	64.8%	32.4%	
Feb. 11, "	76.%	19.9%	Total leucocytes 18,700 per c.c.
Severe anæmia, progressive. Death.			

Jan. 19, 1903.	Injected 1 c.c. Streptococcus.
"	" 50 c.c. 1–5000 Formalin.
Feb. 11, "	53.8% Polynuclears. 41.2% Lymphocytes. Anæmia moderate.

Jan. 21, 1903.	Injected 10 c.c. Streptococcus.
"	" 1–200 Formalin.
Feb. 11, 1903.	58.7% Polynuclears. 38.% Lymphocytes. Anæmia. Nucleated red cells present.

A FEW ANIMAL PARASITES SOMETIMES FOUND IN MAN.

HARLOW BROOKS, M.D.

It has occurred to me that it might be interesting to some of you to have your attention called to certain parasites found in the lower animals which are also found in

man, though generally less frequently. My observations have been somewhat limited and have extended over two years, yet I have found many examples of parasites in the lower animals, which though they are found somewhat rarely in man are oftentimes of the greatest clinical importance. My observations have been entirely limited to the parasites inhabiting wild animals in captivity. Although my experience with wild animals in the open has been rather limited, I feel perfectly justified in asserting that as a rule parasites are infrequent in this state, but that when these beasts are confined, particularly when they pass through the generally filthy and infected quarters of the average animal dealer, they receive most of the parasites which we find so commonly in them in captivity. It is also true that they often receive their infection from the exhibition paddocks and cages, generally because of the lack of knowledge on the part of animal men of the importance of cleanliness and disinfection.

There is a very general idea even among medical men than man often becomes infected with parasitic diseases from handling these animals. This is doubtless true in a certain number of cases; but I am convinced that it is by no means so common as is popularly believed, for a very large number of parasites found in the lower animals, although they belong to the same class as those found in man, are quite incapable of infecting man; the genus found in man often differing in small but very important ways.

This point is perhaps best illustrated in the hook worms, many species of which have been described, though in the ordinary text book no mention is made of this fact and the reader is left to infer that the disease may be transmitted from one animal to another or to man. As a matter of fact, this parasite differs in nearly

every variety of animal which it inhabits and for the greater part the worms infecting one family are quite unable to live in other animals and sometimes even in different members of the same family. For instance, one variety of the Anchylostoma duodenale is found infesting the intestines of dogs and a quite distinct species is found among the foxes. One variety is found among sheep, another among cattle. A few years ago, Lucus showed that a distinct variety of this parasite was found infecting and causing the death of great numbers of the Alaskan seal pups. It has been quite certainly shown that the variety of the Uncinaria infecting man cannot infect the dog family, though they resemble each other quite closely; the converse is probably also true.

Unfortunately the exact zoological status of the hook worm is still in question, and the matter is still further complicated by the fact, as stated by Stiles, that the disease as found in man is known by some fourteen different names, the most common of which are perhaps Anchylostomiasis, Egyptian Chlorosis, Tunnel workers' disease, and, most correctly, Uncinaria.

The lesions produced by all members of the large family of the hook worms are practically the same in all animals. Briefly they consist of the lodgment of the parasites in the duodenum chiefly, where they attack the mucous membrane of the gut—the worm being a blood sucker. From the lesions of the mucosa from which hemorrhage subsequently follows, as well as a result of the blood actually abstracted by the parasites, serious and often fatal anaemia results. Stiles in a recent contribution from the Bureau of Animal Industry, points out that the anaemia is further accentuated by a limitation of the absorptive and digestive surfaces of the gut, on account of the lesions of its mucosa. Some authors also assume that

the parasite forms a poison which also increases the blood destruction. I think this rather doubtful.

The disease has long been recognized in Europe and in the tropical countries; but cases are not frequently reported in American literature, probably due to the fact that heretofore the disease has been rare in those portions of the country where medical investigations are most actively carried on. Undoubtedly the disease has been endemic in the states bordering on the Gulf of Mexico and in other Southern States as shown by Stiles.

Capps, of Chicago, in the Journal of the American Medical Association of January 3, has collected fifty-one cases reported in American literature. Ten of these cases were reported from New York State; but in nearly all instances they were cases of imported infection, either from the tropics, from Europe, or from the Southern States.

The subject is of the greatest importance to us just now, particularly in New York City, since soldiers and civilians returning from Porto Rico and the Philippines are bringing the infection home with them. Furthermore, it is well recognized that the disease is generally spread among earth workers; hence with the extensive tunnels of our underground system and the tunnel which the Pennsylvania Railroad is to build, we are practically certain to find the disease common in New York within the next few years. The gravity of the situation renders wide dissemination of the knowledge of its dangers necessary.

Two varieties of the parasite are found in man. One is known as the Old World hook worm and is generally found in Europe, especially in the warmer parts. The New World species, described first by Stiles, and known as the Uncinaria Americana, is found in Porto

Rico, in our Southern States, and in the West Indies generally. In Cuba, however, both species are found. The observations of Capps seem to show that Panama is infected by the Old World species, hence we may safely assume that both varieties will be found in New York, on account of the migratory character of the laboring classes found here. The two varieties differ from one another in the arrangement of the buccal spines, in the situation of the vagina in the female and in the arrangement of the bursa copulatrix of the male.

I have two sets of specimens to show: the first were taken from the duodenum of a coyote which was one of several to die from the disease at the New York Zoological Park. The animals were sick for some weeks; they became very much emaciated and anæmic. The stools became dark in color and sometimes bloody. Post mortem, a general anæmia of the viscera was found, and in the duodenum large amounts of blood clot were present. On removing this clot, numerous anchylostoma were found, for the most part adherent to the bleeding points of the mucosa. The bleeding points could be easily detected even though the worm had left, perhaps to attach itself to some other part of the intestinal wall. Occasionally the parasites were found coiled up in a small excavation in the mucous membrane or in the mass of clot surmounting it. It is probable that the animals brought the infection with them from the western plains where they were captured.

My second series of specimens was given to me by Prof. Dunham who received them from a case of human infection in Porto Rico. The parasites in this case are undoubtedly the true Uncinaria Americana of Styles. The variety found in the coyote is probably the Uncinaria Stenocephala. The chief distinctions are to be

found in the fact the parasite in the coyote is somewhat thicker and larger than that of man, and the bursa of the male is larger and more symetrical than that of Uncinaria Americana. The arrangement of the buccal spines also differs. It does not seem necessary for me to go into a description of the parasite in this very brief paper, for all text books describe it fully though only the more recent distinctly distinguish between the human parasite and the varieties found in various animals. Still more rarely do they distinguish between the Anchylostoma duodenale, or the Old World parasite, and the Unicinaria Americana of Stiles. I should especially like to call the attention of those interested to the very satisfactory report of Stiles, who considers the matter in full. His report is published by the Bureau of Animal Industry. The late article of Capps is also complete, though brief.

Another parasite found both in man and in animals which in certain cases gives rise to very serious disease is the Balantidium coli. During the past year I found this infusorium to be the cause of a fatal form of ulcerative dysentery which carried off eventually the entire orang family at the New York Zoological Park. The lesions produced in each case were limited to the colon and consisted of the formation of ulcers similar in their macroscopic appearance to those of amoebic colitis. The character of these lesions and the course of the disease as we observed it in these apes corresponded very closely to those described by Malmsden as occurring in his original cases in man. In my cases there was no possibility of doubt but that these parasites were the cause of the dysentery and that they produced the lesions found. There has, however, been considerable doubt expressed on this point in regard to the infection in the human. These doubtful statements are generally expressed in

text books which have for the greater part taken their conclusions entirely from the statement of Malmsden in his original article. I believe that I have shown this conclusion to be erroneous, and I am pleased to find the more recent writers, particularly those who have had the opportunity of observing cases, to be of a like mind. Thus, Strong, who observed cases of tropical dysentery in the Philippines where this parasite is not uncommon, came to this same conclusion. I have corresponded with Dr. Strong on the subject and I am quite certain that the parasite found by him in cases of tropical dysentery is identical with that found in my cases. The parasite is doubtless found normally in the intestine of many animals where it lives without exciting any disturbance. It is common in the hog; and I have found it in the feces of the giant turtles from the Galapagos Islands. It was from these animals that our orangs received their infection. Leuckhart found it in the pig in Germany; and Stiles found it in the American hog. Dr. Russell tells me that he has found the Balantidium coli and also the Balantidium minutum in the feces of dysenteric cases among the soldiers in Porto Rico; but he was unable to satisfy himself as to its pathogenicity. The infection is most frequent in man in Scandinavia, where owing to local conditions it is supposed to be generally communicated to man by the pig. Consequently most of the literture on the subject comes from Scandinavia. The more recent articles of which I have been able to receive word are almost unanimous in pronouncing the infusorium a distinctly pathological agent, bearing a direct relation to the causation of this type of dysentery. Thus, S. E. Henschen, in a recent number of the *Archiv für Verdauungskrankheiten*, reports two new cases of the disease in addition to those which he previously reported

in conjunction with Waldenstrom, and at the same time he states very vigorously his belief in the pathogenicity of the parasite. Dr. N. S. Soloveiff, in *Vratch* of March 30, 1902, also reports a new case, and restates most emphatically his belief in the pathogenicity of the Balantidium coli. Thus nearly all the more recent observations of actual cases of the infection tend to corroborate my conclusions. For the reasons stated in my original article, I do not think that many of the previous observations are trustworthy as to conclusions, since they have for the greater part been based on faulty observations.

The importance of this parasite to us at present lies chiefly in the fact that it is being introduced into this country by many of the soldiers returning from foreign service, and suffering from the tropical types of dysentery. Even the most sceptical observers admit that it is not found in man except when associated with severe intestinal disease. Dr. Poor of Carnegie Laboratory has recently found the parasite associated with the amœba coli in a case of recurrent tropical dysentery. In so far as present observations go, this parasite is apparently transmitted from the lower animals to man, where it is probably more pathogenic than in any of the lower animals, excepting the higher members of the primates.

Last year at one of the meetings of this Society, Dr. Miller and I reported the case of an elk which had died from the results of the presence of the *Miescher'schen Schläuche*, commonly called Rainey's Corpuscle, in the heart muscle. At the time we stated that the parasite had been found rarely in the human, though it is common in certain of the domestic animals used frequently for food, particularly in sheep. The most prominent authority on meat inspection, Ostertag, who is a professor in the University of Vienna, recommends that flesh con-

taining this parasite be not excluded from use as human food, presumably basing his conclusions on the rarity of the disease in man and on the fact that Pfeiffer and others have been unable to infect animals experimentally by feeding them with infected flesh. These experiments have now been overthrown by those of Theobald Smith, published in the November, 1901, number of the *Journal of Experimental Medicine*. Smith has been able to infect mice by feeding them the muscle in which the parasite was found.

Very little seems to be known in regard to the life history of this sarcosporidion, even by the veterinarians who come in contact with the parasite very frequently. There can be no doubt but that it is transmitted from animal to animal and probably through intestinal infection. Recently I have been called upon to give some attention to the subject since we have found that at least a large part of the elk herd at the Zoological Park have been infected with the parasite. Eventually it causes death in these animals though it is generally supposed to be almost entirely innocent. Doubtless the reason that it is so fatal with the elk is that, following the observations made by Darwin, diseases especially of an infectious nature are apt to be particularly virulent in species unaccustomed but susceptible to the infection. I have every reason to believe that the disease is extremely rare in wild animals in the open, while it is very frequent in members of the deer family in captivity. In our animals, elk, caribou, and native deer, contrary to the general rule as stated by most veterinary and medical authorities, the infection invades the heart muscle in most cases, and it is through the invasion of this viscus that it causes the death of the animal. It apparently rarely sets up a true myocarditis, but in the several cases which I have studied

causes a sort of parenchymatous degeneration of the heart muscle, which is sometimes accompanied by fatty degeneration and infiltration. I have become convinced from the character of the disease as we have seen it at the Park that it is transmitted from animal to animal, probably through infected soil on which the grass they eat grows or through the supply of water.

I wish to state again my thorough conviction that although the parasite is rare in the human, still I believe that it can be transmitted through the ingestion of infected meat and I think that it should be more generally known that it is frequent in meat and that flesh containing it should be thoroughly cooked before it is used for food. I have been somewhat surprised to find in talking with some meat inspectors here in New York, that some of them at least know very little about the parasite, and are I believe quite unable to recognize it.

Cases of human infection have been reported by Kartulis, Barbaran and St. Remey, Rosenberg, and Lindermann. Leuckhart, on the other hand, states that it has never been found in the human. The parasite certainly belongs to a very large class and since the life history is unknown, we must accept somewhat carefully statements that the forms found so rarely in the human are certainly those frequent in the lower animals.

Discussion.

Dr. J. C. Roper said that at the Hudson Street Hospital they had had recently two cases of infection with Anchylostoma. The patients were father and son. They had entered the hospital with marked anæmia, twenty-five to fifty per cent. eosinophilia. On examination of the stools from the father anchylostoma were

found. In the son's stools only eggs were found, until after the administration of an anthelmintic. There had been no diarrhœa. During treatment they markedly improved. In both of these cases the eggs of the Trichocephalus dispar were also found in the feces.

Dr. W. B. Noyes said that he had lately been asked to look at the feces of an Italian boy of sixteen at the Columbus Hospital. The boy had been in the hospital for some weeks. He was very anaemic and had had occasional diarrhœa, but had not at any time been very sick. At one time a provisional diagnosis had been made of tuberculous peritonitis. On examination the feces were found to be filled with the ova of some parasite which were apparently identical with anchylostoma. There were a few, however, which resembled the Trichocephalus. A diagnosis of Anchylostoma had been made by one of the attending physicians on the strength of the anaemia and the obscure intestinal lesions. The boy had been in this country for some time. Dr. Noyes said that the ova were identical in appearance with the pictures of the Anchylostoma ova, and though the history was obscure he presumed that this had been a true case of Anchylostoma, though the diarrhœa had not been marked and the child had not recently been in a tropical country.

Dr. A. E. Thayer asked Dr. Brooks whether there were any distinct clinical symptoms by which the disease induced by the Miescher's Schläuche could be recognized.

Dr. Brooks said that the clinical symptoms in the animals were rather striking. There was marked anaemia and disturbed circulation. The beasts lost weight rapidly and became very weak. In humam infection the diagnosis was more difficult and probably could not be made out. The important point which he wished to

raise was that meat which contained these parasites was not fit for use unless thoroughly cooked.

Dr. L. B. Goldhorn said that as regarding the question of recognition it might be of interest to draw attention to the fact that the ova of Anchylostoma were more easily recognized than those of any other intestinal parasite. It was only necessary to set the feces aside for one or two days, when the ova would be found to undergo segmentation.

Dr. Roper said that in one case he had watched development of the ova beyond segmentation. They had had specimens of the large worm and also of the ova. The patients in these cases had come from Brazil.

Dr. Brooks said that the ova sometimes developed in the female before their discharge. Segmentation in the oviducts was rather common. He had seen this in the parasites from animals and it had also been seen in human parasites.

A CASE OF FILARIA BANCROFTI.

F. TILDEN BROWN, M.D.

Dr. Brown said that, associated with a mistake in clinical diagnosis, he had had the good fortune, on microscopical examination following operation, to find numerous embryos, in different developmental stages, of what he took to be Filaria Bancrofti. Although subsequent examinations of the patient's blood had failed to show the embryos, he thought this might in part be explained by the supposition that the orchidectomy had removed all the adult female worms, if in fact more than one had been originally present. In such a circumstance, it was a question of some interest how long the immature para-

sites which had already been discharged into the circulation might be expected to persist. The microscopic slides showing numerous embryos and ova were presented as well as micro-photographs of the same made by Dr. Sondern. The history of the case in which these worms had so unexpectedly been found substantiated what had just been said by Dr. Brooks, that with the extension of the interests of this country in tropical lands we must be prepared for manifestations of new diseases resembling perhaps quite closely other diseases with which we have long been familiar. The patient, a German, thirty-four years old, who as naturalized citizen had been in the United States Marine Service and had been stationed at Porto Rico for the past two years, had had no fever or illness of any kind. During the past two months he had first noticed an enlargement and slight tenderness of the left testis. The possibility of its being a barrier to re-enlistment had led him to enter Bellevue Hospital for treatment. On examination, Dr. Brown had found a moderate enlargement of the left testis. It had been difficult to determine whether a slight but tense effusion in the tunica vaginalis was responsible for the symmetrical increase size or not. A small nodule, the size of a marble, slightly tender, was felt where the mid-body of the epididymis is apposed to the testis, which of the two organs it involved could not be determined. The lower part of the spermatic cord was larger than its fellow. This vas was also thicker but not beaded. The patient had had no gonorrhœa for ten years and never a swollen testis from this cause or from traumatism. A diagnosis was made of tuberculous epididymitis. The patient requested removal of the testis. At operation, nearly an ounce of straw colored fluid occupied the tunica vaginalis. This sac together with the testicle, lower third of the

cord, and considerably more of the vas was removed. Palpation of the specimen now showed that the small nodule occupied, not the epididymis, but the rete testis just underlying it. On cutting into the lesion, a compact fibrous periphery was noted, and in its center yellowish white semi-fluid material somewhat resembling tuberculous debris. The knife was drawn sideways over this surface. The small quantity of adherent material Dr. Brown shortly after transferred to a couple of microscopic slides. On examining one of these which he had stained for tubercle bacilli, some worm-like forms were seen. The other slide had been treated with eosin glycerin and showed a number of field clusters of lumbricoids having the characteristics of Filaria sanguinis hominis. The larvae were distinctly granular and surrounded by the loose fitting hyaline membrane. The immature forms were of oval shape, and intermediate forms showed the embryo coiled and doubled in a larger oval sac, while those more advanced presented the elongated and undulating shapes seen when these parasites are found in the blood. Dr. Brown concluded that in cutting into the lesion he had opened a dilated lymph vessel or space, the abiding place of an adult female filaria; the knife had probably severed this worm, perhaps in several places, and on scraping it sideways the uterine canals had been compressed and more or less emptied of their contents, in this way accounting for the great number of embryos found in different developmental stages. The testicle and its attachments had unfortunately already gone to the pathological department before Dr. Brown had discovered its unexpected and unusual nature, and by the time word was sent to Dr. Goldhorn asking to have careful search made for the adult worm or worms the specimen had been considerably cut and placed in

formalin solution. Many of the clinical features of this case were not unlike that so well presented in the *American Journal of the Medical Sciences* for November, 1900, by Drs. Howard A. Lothrop and Joseph H. Pratt, of Boston. In their case they had the good fortune to recover seven female and two male Filaria Bancrofti from the lymphatics of the epididymis.

A SIMPLE AND RAPID CHROMATIN STAIN FOR THE MALARIAL PARASITE.

FRANCIS CARTER WOOD, M.D.

The recent increase in our knowledge of the alterations in the morphology of the blood which occur in disease is largely due to the simplification in the technique of staining which has been so pronounced in the last few years. As a result of this technical simplicity the clinician has at his disposal the means for the rapid and easy preparation of suitably stained blood smears, and in consequence a large amount of material has been observed and recorded. Believing that any improvement in the technique in blood staining which tends toward ease and simplicity of manipulation may be of use to students and teachers of blood morphology, the writer has felt that certain methods which he has found valuable may not be without interest.

The first experiments were based upon an accidental observation that old alcoholic solutions of the eosinate of methylene blue in the form devised by Jenner (1), and republished as an independent discovery by May and Grünwald (2) three years later, are capable of giving a

fairly good chromatin stain in the young forms of the malarial parasite. The methylene blue component of the dye is apparently altered by the alkali dissolved from the glass of the container and a portion is transferred into what has been shown by Michaelis (3) to be closely allied to, if not identical with methylene azure. If a slide containing malarial parasites is stained with this ripened Jenner dye and slowly washed off in distilled water, a moderate chromatin stain is produced showing the larger nuclear masses of the ring forms and also the reddish granules occasionally seen in the bodies of the lymphocytes. Such a selective stain is not, however, produced on smears which are dropped quickly into distilled water or washed off under the tap, for the peculiar quality of the stain which causes it to color the chromatic substance appears only when the dye is in a watery solution and depends perhaps upon some phenomenon of dissociation of the components of the salt in water. The alcoholic solution is not dissociated and its pure blue color is immediately altered to a reddish purple by the addition of water with the formation of an abundant precipitate.

A number of observers have devised staining combinations in which advantage has been taken of the solubility of the eosinate of methylene blue and methylene azure in methyl alcohol and the power which the mixture has to produce a chromatin stain when diluted with water, among them Leishman (4), Wright (5), and Reuter (6). The latter uses the alcoholic stain only as a stock solution to be diluted with water before use ; the others fix and stain at the same time in the methyl alcohol in which is dissolved the precipitate formed by mixing eosin and methylene blue, containing some methylene azure produced by previously heating the methylene blue with a dilute alkali.

To obtain a chromatin stain with the Leishman or Wright mixtures it is necessary to dilute the fresh alcoholic dye with distilled water after it has been in contact with the smear for a few minutes and allow this combination to act for from three to ten minutes upon the blood spread, but the precipitate which immediately forms when the mixture is diluted is very likely to be deposited upon the smear and give rise to annoying artefacts which can only be removed by treating the slide with strong methyl alcohol and repeating the staining process. The writer has also found after a considerable experience with both methods that it is difficult to obtain constant results even when using smears of the same age and thickness and the same preparation of the stain.

The Wright stain especially, when it is successful, affords an exceedingly instructive smear and demonstrates the chromatin granules of the malarial parasite sharply and well; but as a stain for general class use or for preparing large series of slides for the use of students, the writer has not found it as useful as a much simpler method, using the purified methylene azure as obtained from Grübler. The use of the pure dye, the preparation of which we owe to Giemsa (7), obviates the uncertain and tedious process of preparing the methylene blue solution by the treatment with alkalies and gives the staining method much of the certainty and simplicity of the old eosin and methylene blue stain so much used before the introduction of the Ehrlich and Jenner combinations. Giemsa and Nocht (8) recommended staining slides for some time in a dilute mixture of the eosin and methylene blue, a method which is necessary for staining old slides but unnecessary for fresh preparations. The writer has found that while this eosin methylene azure combination will stain slides which are from two to three

years old and produce under these circumstances an excellent chromatin stain without any of the diffuse bluish color which is usually seen in preparations of slides over one month old, a fact of considerable advantage especially for the purpose of restaining faded demonstration slides or old type specimens which show some especially intererting features, that an equally good stain of fresh specimens can be obtained as follows:

Fix the preparation for one minute in strong methyl alcohol, wash off in water and stain the slide for a few seconds with a one-tenth per cent. aqueous solution of yellowish eosin. The surplus of the eosin solution should be poured off and a few drops of a one-fifth per cent. solution of methylene azure poured over the slide. The staining will be complete in from one-half to one minute and the slide may be washed with distilled or tap water and dried with blotting paper. A longer treatment with the azure solution produces a deeper chromatin stain, a shorter treatment produces a stain of less intensity. If a stain of any particular depth is required the whole process may be easily observed under the microscope, selecting a group of leucocytes as a guide. The stain will be seen to gradually color the nuclei of the polynuclears a deep red, and at the proper point the neutrophile granules will be easily visible with a good four mm. dry lens. At the same time the nuclei of the large and small lymphocytes assume a deep red color and the cell body of the large forms color a pale blue, while the body of the small cells will be a deep blue. At this stage of the process the malarial parasite is of a pale blue color with deeply red chromatin granules. If the methylene azure has remained in contact with the smear but a short time the red cells will be of a bright pink color, but if the straining is prolonged the red cells become bluish or greenish in color. After

prolonged staining with the azure the color of the neutrophile granules generally fades, and the bright pink of the eosinophiles is replaced by a bluish tint. For general purposes, therefore, it is best not to stain the slide too long with the methylene azure. By increasing the strength of the azure solution up to half of one per cent. the staining process with that substance may be shortened to a few seconds, but overstaining is much more likely to take place. If the blue color of the red cells is very marked, the excess may be removed by treating the smear for about one second with dilute alcohol, say of about 80%, and then washing off in water. Some color is likely to be removed from the nuclear chromatin by this process of discolorization, and the red of the finer chromatin particles of the gametes loses its sharp outline or may be entirely extracted, so that the alcohol treatment is not to be recommended for general use. Thus, by the use of this process a good chromatin stain can be obtained in three minutes after the blood smear is secured. Such preparations may also be used for differential counting, though for general purposes the Jenner mixture is preferable, because the eosinophile and basophile granules are on the whole more characteristically stained. The large and small lymphocytes on the other hand are better demonstrated with the methylene azure. The large mononuclears cannot be constantly separated from the large lymphocytes by any of the dyes containing methylene blue and are best classed with the large lymphocytes. Beginners may mistake the blood plates, which often lie in depressions in the red cells, for malarial parasites, especially as they stain red with the chromatin dyes. The distinction must be made by the differences in morphology. The blood plate is not sharply outlined and the nuclear substance is in the form of an indistinct

mass not surrounded by a clear zone, while the small ring form of the malarial parasite is very sharp in its outline as is also the chromatic substance, and the latter is surrounded by a clear zone and beyond this by the blue ring of the body of the parasite.

The staining of old preparations cannot be achieved in a satisfactory manner with any of the usual blood stains except the Ehrlich triacid which does not demonstrate the malarial parasite. By the use of dilute mixtures of eosin and methylene azure, as suggested by Nocht, it is possible to obtain perfect stains from slides which are months or even years old. The older the slide the more dilute the stain must be and the longer it must remain in the staining fluid. Two methods may be used. The slide should be fixed for at least half an hour in methyl alcohol. Then it may be allowed to remain for from four to twenty-four hours in a strong mixture containing about fifty c.c. of a one to a thousand methylene azure solution to which has been added from two to five c.c. of a one to a thousand aqueous eosin. The mixed solutions are best placed in a Coplin jar and the slides allowed to stand vertically. As a rule a precipitate is not formed but if any should form it is not likely to by deposited on the surface of the smear. In case such a precipitate should be deposited on the smear it can be removed by dipping the slide in strong ethyl alcohol for a few seconds and then washing off in water. Slides stained in this manner are usually much over-colored and must be decolorized in 80% ethyl alcohol and then washed in water. By this means the excess of the blue dye is removed and the chromatin particles in the malarial parasites are well brought out. The same result may be obtained by staining for from twenty-four to thirty-six hours in the above mixture diluted about five times with distilled water. It

is not usually necessary to decolorize slides stained in this dilute mixture. The more concentrated solutions when once mixed can be used to stain four or five series of slides, after which the color is exhausted.

BIBLIOGRAPHY:

1.—L. Jenner: *Lancet*, 1899. Vol. I. p. 370.
2.—May and Grünwald: *Cent. f. innere Med.* Bd. XIII, p. 265, 1902.
3.—L. Michaelis: *Cent. f. Bakt.* Bd. XXIX, 1901, p. 763.
4.—Leishman: *Brit. Med. Jour.*, 1901 p. 757.
5.—Wright: *Jour. of Medical Research*, 1902, p. 138.
6.—Reuter: *Cent. f. Bakt.* Bd. XXX, 1901, p. 248.
7.—Giemsa: *Cent. f. Bakt.*, 1902 Bd. XXXI, p. 429.
8.—Nocht: *Encyk. d. Mik. Technik*, p. 785.

DEMONSTRATION OF BLOOD SMEARS FROM A CASE OF PSEUDO-LEUKAEMIA.

L. B. GOLDHORN, M.D.

Dr. Goldhorn showed some slides from the case of a child with enlarged spleen whose blood he had been asked to examine. The slides showed very interesting changes in the red blood corpuscles as well as peculiar conditions of the leucocytes present. The anæmia, as a glance at the smears showed, was of a very high grade. There were peculiarly degenerated red blood corpuscles instead of ordinary granular ones. The granules seen in the protoplasm of these corpuscles were identical with those seen in the blasts. A peculiar thing was that while fifteen or twenty mitoses of blasts were rapidly found in one smear, in five or six others taken at the same time, none were found. Dr. Goldhorn had noticed this occur-

rence twice before; once in a case of pernicious anæmia, and again in a case of Hodgkin's disease. Again, the first smears which had been taken a week before showed exquisite mitotic figures in very large red blood corpuscles; on taking blood again he had failed to find megaloblast mitoses in any of the smears. The leucocytes had been extremely interesting. There was a very marked eosinophilia though there had not been time for a differential count. A peculiar constituent of the blood were very large mononuclear leucocytes, the cytoplasm of many of which exhibited a strong affinity for the basic dyes. These cells were very abundant in this particular case. As regarded the leucocytes in detail, it seemed almost impossible to make a differential count because many forms seemed to have left the bone marrow in an unfinished form, hence many leucocytes present could not be classified under the ordinary headings. Myelocytes were also present. From one point of view the blood resembled pernicious anæmia, while from another point of view it resembled spleno-myelogenous leukæmia.

Discussion.

Dr. Eben Foskett, in giving the history of the case described by Dr. Goldhorn, said that the patient had first come under his care through his taking up the practice of another physician, Dr. Joseph E. Messenger. Dr. Messenger had first seen the child about the first of January. It had then been ailing for several months, was very anæmic, and had an enlarged spleen. The abdomen was much distended and the child was in a worse condition than when examined by Dr. Goldhorn. He had it on tonic treatment; iron largely, quinine occasionally, with intestinal antiseptics. Dr. Foskett had

first seen the case two weeks before. At that time the child was suffering from acute bronchitis. There was very marked distension of the abdomen; the liver was not enlarged. Calomel was given freely and the next day it was possible to outline the spleen. The spleen extended within one inch of the umbilicus and lower than the crest of the ilium. The child was twenty-eight months old. Its general appearance was pretty good, although it was still anæmic. At the time of the report, the lungs had cleared up from the bronchitis and the temperature and pulse were normal. There were several small glands noticeable in each axilla and also in both groins. Dr. Foskett had after one or two examinations thought that the size of the spleen had slightly decreased, but since that time it certainly had not. The edge of the spleen was very sharply defined. The liver was not enlarged, but the intestinal condition was very marked. Another point of interest was that the mother of this child had had eight or nine children, two of whom had died at about this age and with precisely similar symptoms and history.

Dr. F. C. Wood said, in connection with Dr. Goldhorn's remarks on the irregular appearance of large numbers of mitotic figures in the blood at varying intervals, that he had an interesting case several years ago at St. Luke's Hospital, in a man suffering from pernicious anæmia. The blood taken five days before death, at a time when the patient was presumably moribund, showed an enormous number of mitotic figures and megaloblasts. Often two or three mitoses could be found in a single field of an oil immersion lens. In order to obtain more specimens of this unique blood, the speaker had made a large series of slides on the day following, but on examining these slides very few megaloblasts were found, and

no red cells in a state of mitotic division. The patient recovered from the coma in which he had been on the previous day and improved considerably, so far as his general weakness went, but died suddenly four days later. The autopsy revealed megaloblasts and a typical pernicious anæmia, with red marrow filling the shafts of the long bones. In this marrow were a very large number of large megaloblasts, many of them showing division figures. Such blood crises were not unknown in chlororis, but did not seem to be frequent in pernicious anæmia.

THE PERIOD OF DEVELOPMENT, THE TIME OF GREATEST ACCUMULATION AND THE PERSISTANCE OF DIPHTHERIA ANTI-TOXIN IN THE BLOOD OF A SERIES OF 100 HORSES.

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The maximal capacity of horses to develop antitoxin varies very greatly and depends to a certain extent only upon the amount of toxin injected and the frequency of the injections. This variation is of interest both to the biologist and to the producer of antitoxic sera for medical purposes. The following statements and tables are based on the experience derived by us from the injection of about 200 horses with diphtheria toxin.

There seems to be a maximal antitoxic limit for each horse beyond which it cannot be pushed no matter what the amount of the diphtheria toxin injected. After reaching this maximal limit, which by our methods is attained on the average four months after the toxin injec-

tions begin, the blood serum may continue for a while, if the injections are continued, to contain antitoxin of the maximum amount, but sooner or later the quantity of antitoxin surely diminishes. It is seldom that a horse whose power of developing antitoxin, after having been pushed to the maximum, has diminished, can be stimulated to develop again the maximum amount of antitoxin. The effect of a long rest in the pasture on a good antitoxin horse whose power of developing antitoxin has been run down has been tried by us. Such horses rarely produced again their former maximum amount.

The Health Department of the City of New York has from March, 1897, until May, 1902, made use of 100 horses for the production of diphtheria antitoxin. A consideration of the earlier horses is omitted because the methods used to develop the antitoxin were then variable.

These horses were carefully selected, and were all young, healthy animals, of medium grade, and did not represent any special breed or locality.

In the majority of the horses the toxic effects of the first three or four toxin doses injected were more than neutralized with diphtheria antitoxin. The size of the first injections varied considerably, the earlier horses receiving 300 to 1500 guinea pig fatal doses of toxin, with but little antitoxin, the latter horses receiving 5000 to 40,000 guinea pig fatal doses of toxin fully neutralized. With each succeeding injection the antitoxin amount was gradually diminished.

The toxin injections were increased each time by as much as was considered safe, usually 25 to 50%, and repeated as soon as the temperature fell to the normal, about every five to seven days.

The following table is a summary of our data :

TABLE I.—Horses producing Diphtheria Antitoxic Sera. One hundred consecutive Horses Tested.

Number of Horses producing diphtheria antitoxic sera. Maximum strength	Below 300 units per c.c. 51	300 to 400 units per c.c. 12	400 to 500 units per c.c. 11	500 to 600 units per c.c. 9	600 to 700 units per c.c. 7	700 to 800 units per c.c. 7	800 to 900 units per c.c. 1 (horse 182)	1000 to 1100 units per c.c, 1 (horse 112)	1200 to 1300 units per c.c. 1 (horse 137)
Average of guinea-pig fatal doses given to produce maximum strength	666,316*	1,027,067	1,260,000	1,338,666	725,564	1,367,362*	412,000	1,082,500	354,200
Period of injections	3 months*	3.85 months	4.55 months	4.55 months	5 months	4.64 months	2.5 months	10 months	2.5 months
Average length of time horses continued to produce antitoxic sera of 300 units per c. c. or greater	.....	1 bleeding only, except in case of horse 174 which gave 2 bleedings of 300 units per c.c.	2 months	3 months 27 days	6 months 24 days	5 months**	11.5m'ths***	23 months 18 days	5 m'nths**** 6 days
Percent of differ'nt grades of sera	51	12	11	9	7	7	1	1	1

Percent of horses producing serum below 300 units per c.c, ...51
" " " " " over 300 " " 49
" " " " " " 400 " " 37
" " " " " " 500 " " 26
" " " " " " 600 " " 17
" " " " " " 700 " " 10
" " " " " " 800 " " 3

Fourteen of the fifty-one horses recorded as giving sera below 300 units per c.c. died before a test for strength had been made of their sera, but they are placed in the list because at the time of death it was too early to expect a serum containing 300 units per c.c.

* The small amount of toxin injected and the short period during which the injections were carried out were due to our having got rid of the horses when they failed to increase in antitoxin.

* Horse 184 required 5,214,000 guinea-pig fatal doses and horse 193 required, 1,131,500 guinea-pig fatal doses to develop the greatest immunity. The other five horses of the 700-800 unit grade averaged 648,140 guinea-pig fatal doses to develop the greatest immunity. Horses 184 and 193 bring the average of the time required to develop the greatest immunity of their grade up from 3.3 to 4.64 months.

** Two of these horses are still producing a high grade antitoxin.

*** Still producing antitoxin above 300 units.

**** The early death of horse 137 was caused by an accident and was not due to the toxin injections. Horse 137 was still producing high grade antitoxin.

It will be seen by the table that 49% of these horses were capable of developing antitoxic sera of 300 units per c.c. or more, and that 26% developed antitoxic sera of over 500 units per c.c., the so-called high grade sera. As an example of how horses differ in the amount of toxin injection necessary to produce the maximum amount of antitoxin, may be cited eleven horses which failed to develop 300 unit sera, yet received enormous total toxin injections, varying from 1,195,250 guinea-pig fatal doses to 3,005,500 guinea-pig fatal doses, which covered a period of five ot six months ; average four and one-half months ; while after a total toxin injection of 354,200 guinea-pig fatal doses, horse No. 137 developed 1200 antitoxic units per c.c.

The horses incapable of developing sera containing 300 units per c.c. were replaced by others with the hope of getting stronger sera. This, together with the early deaths of fourteen horses, accounts for the short period they were kept and for the smallness of the average of total guinea-pig fatal doses they received.

It is interesting in Table I to note the great variation in the average number of guinea-pig fatal doses and the average period of injections required to produce the maximum strength in the different grades of horses. The figures are somewhat misleading where single horses are concerned, for the reason that they may have been bled and their injections for several weeks stopped before reaching the maximum amount of antitoxin.

A few notes on some of the horses that have given the largest amount of antitoxin may be of general interest :

TABLE II.—Showing the Relation of the Series of Toxin Injections to the Maximal Antitoxic Strength of the Sera Developed by the Horses.

Number of series of diphtheria toxin injections necessary to bring the horses up to their maximal antitoxic strength.		
	400—500 units per c.c.	500—600 units per c.c.
	1st series, 8 horses. 3rd series, 2 horses. 4th series, 1 horse.	1st series, 3 horses. 2nd series, 3 horses. 3rd series, 2 horses. 5th series, 1 horse.
	600—700 units per c.c.	700—800 units per c.c.
	1st series, 5 horses. 4th series, 2 horses.	1st series, 4 horses. 2nd series, 3 horses.
	800—900 units per c.c.	1000—1100 units per c.c.
	Horse 182, 1 series.	Horse 112, 7 series.
		1200—1300 units per c.c.
		Horse 137, 1 series.

The percentages of horses which developed their maximal antitoxic strength after receiving different numbers of series of diphtheria toxin injections are as follows:

Number of horses 37, beginning with these horses in grade 400–500 units per c.c.

After 1st series of diphtheria toxin injections,			59.38%	Had developed their maximal antitoxic strength.
After 2nd series	"	"	16.21%	
After 3rd series	"	"	10.81%	
After 4th series	"	"	8.10%	
After 5th series	"	"	2.70%	
After 7th series	"	"	2.70%	

Horse 112 was one of the best antitoxin producers; but did not attain its maximum until ten months after the first toxin injection.

Had it not been necessary to bleed horse 112 so early and so constantly the point of greatest immunity would undoubtedly have been reached much sooner and with many less guinea-pig fatal doses. For fifteen months after developing its maximal antitoxic strength horse 112 continued to yield sera above 300 units per c.c. The strength of the serum then fell to about 200 units per c.c., above which it did not rise again.

Horse 137, after the initial bleeding (1200 units per c.c.), never gave a stronger serum than 600 units per c.c., but he held at that strength until his death. Two horses not included in this series of 100 horses are worthy of mention. Horse 83, after receiving 361,000 guinea-pig fatal doses, gave a 500 to 600 unit serum (period of injection 2¼ months).

After receiving 2,904,000 guinea-pig fatal doses additional, the serum did not increase in strength more than 100 units per c.c.

After a rest of 1¼ months the horse was given 1,115,000 guinea-pig fatal doses. The strength of the serum rose to 850 units per c.c. As in the case of horse 112, had it not been necessary to constantly bleed horse 83 the maximal antitoxic strength probably would have been raised much sooner. Number 83 furnished antitoxic sera usually above 500 units per c.c., through a period of 22 months.

One of the first horses treated, number 7, was bled at intervals for four years and gave a serum varying between 300 and 500 units. The injections in this horse were not pushed to the limit. The horse is not included in Table I.

We may conclude from the above data that from carefully selected young horses of medium grade:

1st.—That about one-half will develop sera of 300 units strength per c.c. or greater, and that about one-fourth will develop sera of 500 units strength per c.c. or greater.

2nd.—That the average development of maximum strength may be expected three to five months after the first toxin injections, according as to the rapidity with which the toxin is pushed.

3rd.—That horses capable of producing a high grade antitoxin serve a longer period of usefulness than horses capable of producing a medium grade antitoxin.

In conclusion, I wish to thank Dr. William H. Park, Director of the Research Laboratory, for his kindly criticism, and Dr. George P. Biggs, Assistant Pathologist, for his help in supplying me with much of the data.

NOTES OF SYSTEMIC INFECTIONS BY THE STAPHYLOCOCCUS AUREUS.

E. LIBMAN, M.D.

In the following remarks I intend to make a report of all the blood cultures in which I have found the staphylococcus aureus. These investigations extend over a period of five years. It had not been my intention to speak on this subject at the present time, but the scepticism which was manifested at the last meeting of the Society has led me to make this report. During the course of my remarks I shall refer to a number of interesting details concerning the cases under discussion; but

my main object will be to show that in every case in which I found the staphylococcus aureus in the blood during life, the finding was confirmed. I shall not refer at length to the question of whether the staphylococcus albus occurs in systemic invasions or not. Up to the present time it has been my experience that it occurs in the blood only as an agonal invader, as I have not yet had a case in which I have found the staphylococcus albus in the blood during life and been able to confirm the finding by discovering the same organism in any metastatic focus.

It is of course very important to differentiate the staphylococci found in the flasks and plates. Tubes showing staphylococci should be removed from the thermostat after twenty-four hours and observed for a number of days. Sometimes the aureus color does not develop for several days. Furthermore, it is well to trans plant the colonies on to potato, serum-agar, and ordinary agar, particularly if sugar media have been used.

As to the technique, I shall be very brief, as I expect to go into this subject on another occasion. The upper arm is constricted by means of a bandage; the anterior aspect of the elbow is then scrubbed thoroughly for from three to five minutes with green soap and water, then rubbed with ether, alcohol and one to five hundred bichloride. The syringe and needle are boiled at the bedside and the needle is used while still quite warm. If through some delay or difficulty the needle has become cold, it is rapidly passed through the flame to avoid all danger of contamination. Part of the blood is put into flasks and the remainder is plated. The media most commonly used are serum-glucose-agar, serum-glucose-bouillon, and the ordinary media.

Among the following cases there will be found a

number of instances of osteomyelitis. It is essential, of course, to remember that we must look upon the osseous foci in these cases as metastatic deposits, and we must also bear in mind that these cases, particularly of multiple osteomyelitis, are especially seen in children. Jordan has very well called the condition "the septicæmia of adolescence." In other words, children with systemic staphylococcus aureus infection are apt to have the bones involved, whereas adults with a similar infection are more liable to have other forms of metastatic lesions. So that the cases to which I refer of this nature are to be looked upon simply as a form of sepsis, the primary focus of which in many of the instances cannot be determined; and for that reason it is not possible in a number of the cases to report the findings in the primary focus.

I believe it is best in speaking of septic cases, to divide them into cases of local infection without bacteriæmia and cases of local infection with bacteriæmia with or without metastases. However, in describing my cases I shall for the sake of brevity use the word "septic" in its clinical sense. I wish simply to indicate that the patients had suffered from symptoms which are clinically considered suspicious of pyogenic infections.

In the notes on my cases there will be found a number of reports on the bacteriology of the urine. These, while unnecessary in many of the instances to confirm the blood culture, are of great interest in demonstrating one of the methods by which bacteria leave the body. In a few of the cases, besides finding the staphylococcus aureus, we also obtained a few colonies of albus. To the latter no importance must be attached as it is a normal inhabitant of the urethra. In the later investigations a double catheter was used (according to Melchior), and with this the results were more satisfactory.

The cases of which I wish to present the notes are the following:

Case I., March 13, 1898.—Osteomyelitis of the tibia; culture, staphylococcus aureus; two days later the blood showed staphylococcus aureus. The patient died three days later, and at the autopsy, made ten hours post mortem, the staphylococcus was found in the heart blood, in an empyema, in infarctions of the lung, in an area of pneumonia, and in the abscess of the kidney.

Case II.—This case was reported in detail by Dr. Brill and the writer several years ago. The first blood culture showed the staphylococcus aureus; the second, blood culture made several weeks later (two days before death) showed the bacillus pyocyaneus. At the post mortem examination the staphylococcus aureus was found, in a metastatic abscess, in the right vastus externus muscle, and the bacillus pyocyaneus in the viscera.

Case III.—Staphylococcus aureus isolated from osteomyelitis of the tibia; three days later the blood showed staphylococcus aureus. Staphylococcus aureus cultivated from the heart blood and from miliary abscesses of the lung, post mortem.

Case IV.—Case of carbuncle of the neck with supposed diabetic coma. Blood culture, staphylococcus aureus. Post mortem, staphylococcus cultivated from miliary abscesses of the lung; organism also found in spreads.

Case V.—March 30, 1899.—Staphylococcus aureus reported as being present in the blood. The patient was operated upon the next day for osteomyelitis of the femur; staphylococcus aureus found in the bone focus and again in the blood on the same day.

Case VI.—This case is one of the most interesting in the series. The primary focus was an osteomyelitis of

the femur. The culture sent down from the operating room showed no growth. (This observation is of no significance, as the cultures at the time were usually made by an inexperienced helper in the operating room, who either used the loop too hot or did not succeed in planting the material on the agar.) The blood on the day of admission showed the staphylococcus aureus; two days later the same finding was made; three weeks later the urine showed a pure culture of staphylococcus aureus.

On two occasions after that it showed no aureus. Suspecting that there might no longer be bacteria present in the blood, a blood culture was made several days later and the blood was found to be sterile. The patient recovered.

Case VIII.—Staphylococcus aureus found in the blood and in an osteomyelitis of the left tibia, also in some pus in the right ankle. Post mortem, staphylococcus aureus in the spleen, lungs, and heart blood.

Case VII.—Staphylococcus aureus present in the blood, also present in metastatic foci in the skin. A culture from the foci in the femur and tibia showed the same organism. Patient died; no autopsy.

I wish here to refer a little more in detail to the cutaneous lesions which I have seen in a number of cases of osteomyelitis due to the staphylococcus aureus. I have not seen them with other infections, although there is no reason why they should not occur. These lesions may be manifested over the skin of almost the entire body, but are particularly apt to occur in the scalp. They consist of numerous miliary abscesses in the skin with little or no inflammatory reaction. I have not seen similar lesions in any other form of disease. In every case in which they have been noted there has been present a bacteriæmia.

Case IX.—Staphylococcus aureus found in the blood; latter in the bone marrow of the femur and in the lesion in the scalp and right leg. Patient died; no post mortem examination.

Case X.—Case of osteomyelitis of the femur. Staphylococcus aureus cultivated from the blood and from a metastatic abscess of the side of the face. Patient died; no post mortem examination.

Case XI.—Osteomyelitis of the tibia; staphylococcus aureus obtained on two occasions from the blood and from a focus in the tibia. Two weeks later pus was aspirated from the pericardium which contained the staphylococcus aureus pure. Patient died; no post mortem examination.

Case XII.—Case of post-partum sepsis. It is a common belief that these cases are always instances of streptococcus infections. In the literature there are reported five or six cases in which the staphylococcus aureus was the infecting agent. In my series I have two such cases. In this case the staphylococcus aureus was cultivated from the blood on January 30, 1901; a week later it was again found in the blood; still one week later the urine was examined and found sterile. A blood culture taken on the following day remained sterile, and the patient recovered.

Case XIII.—Staphylococcus aureus found in osteomyelitis of the tibia on February 16, 1901. Five days later the same organism was found in the blood and three days later in the urine. Twelve days later the same organism was found in another focus in the tibia. A blood culture made the next day remained sterile. Patient recovered.

Case XIV.—A case of osteomyelitis of the right femur. (Patient came in too bad a condition for opera-

tion.) Hundreds of colonies of the staphylococcus aureus were isolated in five c.c. of blood and the same organism was found in pus aspirated from an abscess probably arising in the thyroid. Patient died; no post mortem examination.

Case XV.—Staphylococcus aureus found in the blood and in large area of cellulitis in the right arm. Patient died; no post mortem examination.

Case XVI.—Staphylococcus aureus found in the blood eight days before death and again seven days before death. On post mortem examination a fibrinous area found on the wall of the right ventricle showed staphylococci in spreads and the staphylococcus aureus in pure culture. The same organism was isolated from miliary abscesses of the heart wall, kidney, and from infarctions in the spleen. It was also present in spreads and cultures in submucous abscesses of the colon.

Case XVII.—Staphylococcus aureus found in the blood; later in metastatic abscess of the thigh, and in the urine. Two weeks later blood culture negative. Recovery.

Case XVIII.—Staphylococcus aureus found in he blood, in osteomyelitis of the humerus, and in scalp lesions. Patient died; no post mortem examination.

Case XIX.—This patient was admitted suffering from sepsis supposedly secondary to an abscess of the foot. On the day after admission one colony of staphylococcus aureus was found on one of the plates. (Twelve c.c. of blood had been obtained; seven c.c. being used for the flasks and five c.c. for the plates.) Subsequently the staphylococcus aureus was isolated from a metastatic abscess over the left trochanter, from the urine, and from an abscess in the right vastus externus. The patient was subsequently operated upon for an abscess of the right

kidney and the same organism obtained. Patient died; no post mortem examination.

Case XX.—Staphylococcus aureus found in the blood and in an osteomyelitis of the humerus.

Case XXI. This was one of the cases of endomyocarditis shown at the last meeting. The first blood culture was negative. In the second blood culture fifteen c.c. of blood were used and the staphylococcus aureus was found in one of the flasks. At the post mortem examination the staphylococcus aureus was cultivated from the heart blood and from the centre of the vegetations.

Case XXII.—Case of post-partum sepsis. Staphylococcus aureus found in the blood the day after admission, and two days later found pure in the urine. Subsequently it was isolated from a pus kidney on the right side and again from the urine. A later blood culture was negative. Patient recovered.

Case XXIII.—Staphylococcus aureus found in the blood and later in an abscess over the left hip joint. Subsequently it was found in the urine twice and in the blood once. At the post mortem examination the organism was isolated from an abscess of the sclera of the left eye, from a periarticular abscess over the shoulder, from an abscess cavity in the dorsal region leading to the vertebræ, from an abscess under the left pectoralis major, from a purulent pericarditis and from purulent infarcts of the kidney.

In these twenty-three cases in which I have found the staphylococcus aureus in the blood during life the finding has been confirmed in every instance. In three hundred and thirty other blood cultures I have never found a single colony of the staphylococcus aureus. I think, then, that as far as these cases are concerned

there can be no doubt as to the validity of the blood findings.

It will be noted that out of the twenty-three cases five recovered. It is generally considered that the more bacteria there are found in the blood the less chance there is of recovery. This does not necessarily hold good. The number of bacteria found depends, in some cases at least, upon the time at which the examination is made, as the great tendency is for the body to rid itself of bacteria and for metastases to be established. The blood cultures in the fatal cases under discussion were made at periods varying from one to nineteen days before death. The reports could usually be handed in within twenty-four hours. Sometimes colonies were seen after six or eight hours. In some instances the report was delayed several days as the organism was very slow in developing its typical color.

I wish to thank the members of the medical staff of the Mt. Sinai Hospital through whose great kindness I have been enabled to cary on these studies.

Discussion.

Dr. W. H. PARK said he thought this report gave a feeling of confidence in the results of blood cultures when properly made since Dr. Libman had proven so many of his positive results by his findings of the same organisms after death, in the primary lesions.

Dr. P. H. HISS asked Dr. Libman for a little more detail in regard to the scalp infection of which he had spoken. Were these abscesses deep or superficial?

Dr. LIBMAN said that some were deep and some were superficial. They did not seem to correspond to the hair follicles. He was inclined to look upon them as being dependent upon the attempt of the skin to eliminate

the bacteria. He had not introduced them so much to confirm his blood cultures, but rather as a matter of clinical interest.

Dr. Hiss said that personally he would be inclined to think that they were secondary infections, due to the general systemic resistance having been lowered by the disease, and were probably not caused by the original infecting organism, but by organisms of the same species already present in the follicles.

Dr. Cyrus W. Field said that he had looked over the records at the Presbyterian Hospital of five hundred cases in which blood cultures were taken. There was one case which was very interesting. Eight tubes had been taken; four in bouillon and four in blood serum. Of these, two in blood serum and three in bouillon developed staphylococcus aureus. The case had turned out to be a typical case of typhoid fever and typhoid bacilli had been isolated from the blood, showing that it was undoubtedly not a staphylococcus infection. The staphylococcus had been in the skin and had contaminated the needle, thus being introduced into the culture media. As the staphylococcus grows rapidly on blood serum and bouillon, Dr. Field thought he would doubt the presence of the organism unless it was found in all tubes. He thought there was a great deal of doubt as to the staphylococcus aureus being constantly found in the blood. He considered it a frequent contamination, and that when it was present in cultures great care should be taken in interpreting the findings. The point raised referring to the urine had interested him very much.

Dr. Hiss said that Watson-Cheyne had been the first to call attention to this question of the excretion of staphylococcus aureus through the intact kidney epithelium, and considered that the abscesses then arose

from proliferation of the organisms in the tubules. The speaker had not placed very much confidence in the excretion of organisms through the intact epithelium, because in the experiments to recover the organisms from the bladder contents the chance of getting the organisms from the blood had seldom been eliminated. When due care had been taken in obtaining the urine specimen for analysis, it was his impression that organisms had seldom, if ever, been found in the urine, unless some pathologic change had occurred, which affected the integrity of the excreting organ.

Dr. James Ewing said that during the past year Bertelsman had reported that in *Langenbeck's Archiv* a series of one hundred bacteriological examinations of the blood. Out of this hundred, forty-one gave positive results. Of the forty-one there had been fifteen cases in which the staphylococcus aureus had been found. In looking over the details of this article he had noticed results which were quite contrary to those obtained by others, especially in the large proportion of positive cultures from patients who recovered. In comparing these observations with the work of others he had decided that they required confirmation, especially in cases where the streptococcus was found in osteomyelitis, panaritium, urethral fever, and in puerperal sepsis, and yet the patients recovered. It had seemed to him that these results were contrary to those generally obtained. Dr. Libman's report seemed to give in some degree the confirmation required. He felt no doubt that Dr. Libman's cases of staphylococcus aureus infection were genuine. The large number of cases encountered could be explained by the fact that he obtained his material from a very active surgical service. The scepticism manifested at the last meeting of the Society in regard to the

frequency of staphylococcus aureus in cases of endocarditis he had shared. He had noticed in the report just made that out of a series of twenty-three cases only two were found in malignant endocarditis, and he thought that Dr. Libman must have had a much larger number than two of malignant endocarditis out of three hundred examinations. This did not seem to him to indicate that the staphylococcus aureus had been found in a large number of cases of malignant endocarditis.

Dr. F. TILDEN BROWN asked what Dr. Libman's practice had been in the taking of blood. Was it to collect as much as ten or twelve c.c. or to take lesser quantities for the initial test?

Dr. FIELD said that out of the five hundred cases of which he had spoken only twelve had shown staphylococcus aureus, and but three of these were cases of malignant endocarditis.

Dr. LIBMAN said, in regard to Dr. Hiss' remarks about the scalp lesions, that he himself had never seen the particular type under discussion in any other disease, and would be glad to hear whether any one present had seen the lesions to which he wished to draw attention in cases of osteomyelitis or any other diseases. He thought that the lesions occurred particularly in the scalp in children because of the activity of the sweat glands in that part. Dr. Libman said that there were four channels through which bacteria were supposed to be eliminated from the body, these being the skin, urine, bile and milk. As regarded the milk, the question was still an open one. The excretion through the bile could hardly be studied in the human being. He had introduced the details concerning the skin lesions and the urine to draw attention to two channels of excretion. He said that there was much discussion going on as to whether bacteria

could be eliminated from the kidneys if the renal epithelium and vessels were normal ; but clinically this point was of no importance, because by the time patients came under observation they were all apt to have decided degenerative changes in the organs.

In reply to Dr. Field's remarks, Dr. Libman said that he had been willing to report positive findings even if all the plates and cultures did not show organisms. The cases cited, he believed, proved that he was correct in in this view. Furthermore, he said, he used several kinds of media in each blood culture, and if he obtained organisms in only one flask or one plate it might be that it had only occurred there because he had used a particularly favorable medium in that flask or plate, and that those who used simply one of the ordinary media might easily miss the organism.

Dr. Libman said that he could hardly agree with all the reports of Bertelsman, as he was sceptical concerning Bertelsman's reports of the staphylococcus albus in the blood. Of course, it was possible that he had not sufficiently tested the organisms. He was under the impression that Bertelsman used the insufficient method for disinfecting the skin, which Schottmuller used.

As regarded Dr. Brown's question, Dr. Libman said that the amount of blood used varied from two c.c. up to twenty-five c.c.; as a rule it was five to fifteen c.c. In several cases of plain streptococcus septicæmia he had found the organism in a few drops of blood.

In conclusion, he wished to draw attention to the fact that in blood culture work one must depend almost entirely upon one's own experience, as there were so many conflicting reports in the literature.

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Dr. William H. Park, *President.*

A CASE OF BACTERIURIA RESEMBLING WEIL'S DISEASE.

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The history is that of a male, aged 56. Habits have been non-alcoholic but a fairly heavy eater. He has had scarlet fever. There is no history of syphilis. Five years before this illness he had an attack of acute intestinal toxæmia with temporary paralysis of the lower extremities, vomiting, diarrhœa and a partial loss of consciousness. This followed a large dinner, eaten the night before.

The previous records of his urine are as follows:—

October 29th, 1901, Amber, clear, 1,020, faintly alkaline, no albumin, no sugar, .7% urea.

November 17th, 1901. Amber, clear, highly acid, slight floculent precipitate, no albumin, no sugar, .65% urea, calcium oxalate crystals, no casts. Other records of urine examinations previous to this have been lost, but no albumin nor bacteria to any appreciable extent were present at any time.

For a short time before the present illness, which began November 29th, 1901, patient had slight constipation and flatulence. The night before the attack, he ate a large Thanksgiving dinner. The following evening he had a very severe continuous pain over the lower part of the sternum with some flatulence and general abdominal distension. The next morning the pain was no better, the distension and flatulence were still present. Then followed severe chills with profuse sweating and a condition of shock, slight delirium and stupor. I saw him at six in the evening when he was in a semi-stupor and complaining loudly of pain over the lower sternum. The temperature was 103°, the pulse 120, and the respirations very rapid and labored. The heart's action was tumultuous, and intermittent, with loud systolic murmurs at the apex and base. The second aortic sound was loudly accentuated. At 8 P. M., the temperature reached 105°, the pulse 134 and the respirations 43.

Examination of the lungs showed numerous subcrepitant rales all over the right lung with shallow and deficient breathing, but no other signs. There was no tenderness over the abdomen nor apparent enlargement of the liver. A tentative diagnosis of lobar pneumonia was made and treatment accordingly was carried out.

The tongue was dry, and with the labored and irregular heart's action, the prognosis seemed bad. At

11 P. M., however, the temperature dropped to 103°, the pulse to 104 and the respirations to 32. The heart's action became regular and the general condition was improved.

At 6 A. M. the next day, the temperature, pulse and respirations were normal. The skin and scleræ were deeply jaundiced. A large brown normal stool was passed.

Examination of the urine showed it to be dark brown, cloudy, 1,026, containing 5% of albumin by volume, no sugar. Bile salts are present. There are numerous granular and epithelial casts, all bile stained and numerous bacteria. The highest point the temperature reached this day was 100.3° at 9 P. M.

On the third day of the illness, the temperature reached 103°.

The urine was dark brown, cloudy, acid, 1,012, contained 5% of albumin, and 1.6% of urea. There were fewer granular casts than the last time with bile stained epithelium and spermatazoa. The urine was very cloudy directly after passing, due to the very large number of bacteria present.

Cultures were taken from this urine. In the evening, a large, formed, clay-colored stool was passed, showing that there must have been obstruction in the bile ducts.

On the fourth day the jaundice became much deeper.

On the fifth day, the temperature reached 100.2°, after which convalescence was uneventful, but accompanied with severe muscular pains, weakness and indigestion for several months. The albumin in the urine gradually disappeared, until at the end of two weeks none was present except a faint trace, by the heat and acetic acid test. The urine was always very cloudy and the

bacteriuria, constant. This was uninfluenced by the administration of urotropin.

One month later the bacteriuria persisted. Lavage of the bladder had no appreciable effect upon it. The prostate was not enlarged but slightly soft. Secondary infection of the prostate may have had some effect in keeping up bacteriuria. Occasionally for a day or two the urine would be clear on passing and then cloudy again.

Three months later, patient had a somewhat similar attack, without jaundice, however. He has had no illness since then.

I isolated a bacillus from the urine resembling the proteus group. For outlines, see accompanying chart.

The chart shows a comparison of some of the chief characteristics of the bacillus with those of Weil, Jäger, Brooks, Libman, the proteus Zenkeri and the bacillus enteriditis. The two latter are similar bacteria which inhabit the intestinal tract. The colon bacillus is excluded at once. Weil isolated his bacillus from the urine of a case of acute febrile jaundice in 1886.

Jäger found his bacillus in the urine of a case of acute febrile jaundice. He also isolated the bacillus from the bodies of sick fowls whose bodies had contaminated the water supply.

Brooks isolated his bacillus from the organs at autopsy. The patient died with the symptoms of Weil's disease.

Libman found a bacillus in the pus of a liver abscess, that was followed by an acute febrile jaundice.

Jäger's and Brook's experiments were very careful and thorough.

WEIL	JÄGER	BROOKS	LIBMAN	PROT. ZENKERI	BAC. ENTERIDITIS	SATTERLEE
	Motile	Motile	Motile	Motile	Motile	Motile
Similar to Jäger	Bacilli and cocci	Bacilli and cocci Polymorphic, bipolar staining	Bacilli and cocci, size vary	Small bacilli and cocci	Thick, short bacilli.	Bacilli and cocci vary in size bipolar
	No spores	No spores	No spores	No spores	Spores	No spores
	Some liquify gelatin, others, not	No liquefaction	Some liquify gelatin, others, not	No liquefaction	Liquefaction	No. liquefaction
None	Greenish fluorescence on agar	Thick, heavy spreading	Greenish fluorescence on agar			Thick mucoid growth on agar
	Milk, not coag.	No coag.	Coag. in 24 hrs.	Milk coag. slowly	Milk coag. slowly	Milk coag. slowly
	React. unchanged					Slightly acid
	Ferment. marked	Ferment. marked	Ferment. marked	Ferment. present	Ferment. present	Ferment. marked
	Gram decolorizes	Gram decolorizes	Gram decolorizes	Gram decolorizes	Gram decolorizes	Gram decolorizes
	No indol	No indol	Indol present and rapid	No indol	Indol present	Indol present
Changes in exper. animals	Pathogenic to mice and pigeons Acute hepatitis. Fatty degen. of kidney, acute neph. hemorrh. in other organs.	Path. to mice, guinea pigs, monkeys, NOT to rabbits Acute necrotic hepatitis, Acute nephritis, Acute ganglion cell degeneration	Path. to rabbits Fatty degen. and necrosis of liver cells, Acute nephritis, Fatty degen. of renal epith.			Path. to guinea pigs and to rabbits in large quantities, Acute hepatitis, Fatty degen. of liver cells, Acute nephritis, Acute ganglion cell degen. Hemorrh. in other organs.

Cultures were taken from the urine passed the second day of the illness. Twenty-four hour agar cultures show a profuse whitish mucoid growth. Morphologically the bacteria are large bacilli, with heavily stained ends and a faintly stained centre. (Fig. 1) In places they look like large diplococci. (Fig. 2) The stain readily with the aniline dyes. The bacilli have no definite arrangement. No spores at present. The organism is slightly motile.

Superficial agar colonies have a dark yellow brown centre and a light grey fibrous-like border. Deep agar colonies are large, oval or round, yellowish brown and coarsely granular.

Deep gelatin colonies are greyish yellow with lighter border, of a definite smooth contour. Gelatin stabs show a profuse rapid growth with fine arborescent projections. On the surface they are circular and spread out like a tack head. There is no liquefaction.

Growth in broth is rapid and produce cloudiness with a thick, white, mucoid pellicle. There is also a white, flocculent, precipitate. Agar growths are acid in reaction.

The bacillus is a facultative anaerobe. Milk is coagulated slowly, reaction slightly acid after a growth of forty-eight hours. Glucose fermentation is marked, the gas produced containing carbon dioxide but no hydrogen sulphide. Indol is present. The growth on potato is white, thick and slimy and produces discoloration of the medium.

Cultures in normal, acid urine show rapid abundant growth with cloudiness but no pellicle. The urine is acid up to seventy-two hours, after that alkaline.

FIG. I.—SMEAR FROM RABBIT'S BLOOD, SHOWING BIPOLAR STAINING, GOLDHORN'S POLYCHROME METHYLENE BLUE STAINS.

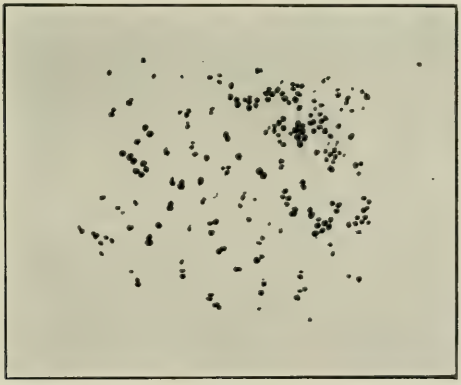

FIG. II.—DRAWING OF BACILLI SHOWING COCCUS AND INVOLUTION FORMS.

Animal Experiments.

I.—A small guinea pig, injected subcutaneously with 2 c.c. of a 48 hour broth culture. The pig died during the night. The autopsy showed intense congestion of all the organs. There was a slight amount of turbid fluid in the peritoneal cavity. Cultures taken from the heart, peritoneum, liver and bladder show pure cultures of the bacillus.

II.—Guinea pig, injected subcutaneously with 1.5 c.c. of a 48 hour broth culture. The animal survived with apparently no bad effects.

III.—Guinea pig, injected intraperitoneally with a 2 c.c. of a 48 hour broth culture. A few hours later the animal became very quite, remained in the corner of its cage and did not eat. It died during the night apparently without convulsions. Autopsy shows absence of rigor mortis. The heart was apparently normal, the ventricles firmly contracted. The abdomen was soft. The peritoneal cavity contained about 5 c.c. of a sero-bloody fluid. The stomach is full of food. The colon is slightly distended with gas. The blood vessels of intestine are congested. The kidneys are congested and oedematous. The liver and spleen are congested. There is no urine in the bladder. The blood vessels of the brain are deeply injected. Pure cultures are obtained from all the organs.

In order to determine the possible effect of toxines, a broth culture of several days standing was filtered and shown to be sterile.

IV.—Guinea pig was injected subcutaneously with 2 c.c. of the filtered culture with a nagative result.

V.—Guinea pig was injected intraperitoneally with 2 c.c. of the filtered culture with a similar result.

VI.—A 1123 gram rabbit was inoculated intravenously with 2 c.c. of a broth culture. Twenty hours later, the animal looked sick and the weight had fallen to 988 gram. No evidences of jaundice were present. The injected ear was œdematous and paralyzed. The animal was catheterized and a specimen of urine obtained.

Examination of the urine showed it to be cloudy, acid, no sugar, a heavy trace of albumin, urea 2.7%. No bile was present. There were numerous hyalin casts and calcium oxalate crystals; numerous bacteria and a few leucocytes. Cultures from this urine show a pure culture of the bacillus.

On the following day the rabbit improves, eats well, and weighs 1028 grams. The ear is still swollen and a patch of dry gangrene appeared. The urine contains no albumin, only a few actively motile bacteria and a few granular casts. Culture again shows the bacillus in pure culture. The rabbit has evidently recovered from the illness caused by the injection into the circulation, and is now suffering only from the local disturbance in the ear. The rabbit made a complete recovery.

VII.—5 c.c. of a two day broth culture is injected into the peritoneal cavity of a 1446 gram rabbit. Six hours later, the animal appears sick and does not eat. It is quite but moves about when excited. The next morning the animal was found dead. Unfortunately parasitic infection of the liver and kidneys obscured the pathological findings.

The results of experiments on animals showed the bacillus to be toxic for guinea pigs and to a less degree for rabbits. The bacillus was recovered in pure culture from all the organs. Filtered cultures of the bacillus produced no effect.

The microscopical examination of the viscera of the guinea pigs showed about the same picture in each instance. The *heart* shows a moderate congestion of the blood vessels, especially of the capillaries; otherwise, normal. There is slight congestion of the *pulmonary* blood vessels.

There is intense congestion of the *liver*, especially of the portal radicles. The liver cells show acute parenchymatous and marked fatty degeneration, most extreme in the central part of the lobule. In places the liver cells are almost completely destroyed. The bile capillaries are much distended and there is marked pigmentation of the liver and connective tissue cells. Apparently a hypersecretion of bile is present.

The blood vessels of the *spleen* are markedly congested. There is recent hyperplasia in the lymph follicles and a marked pigment deposit in all the parenchyma cells.

In the *kidney* there is a general injection of the blood vessels, notably of the Malpighian tufts and of those surrounding the convoluted tubules. The cells of the convoluted tubules are granular and their edges are jagged and irregular. In many cases the entire cell is broken down into fine detritus, found filling to more or less degree many of the tubules. Some of the collecting tubules contain fairly well formed hyalin and granular casts.

The *brain* shows marked injection of the blood vessels. There are hemorrhages into several of the perivascular spaces. There is congestion of the blood vessels with capillary extravasation in the cerebral membranes. The Purkinje cells of the cerebellum show finely granular chromatolysis in many instances.

The microscopical changes in the viscera of the rabbit are very similar but not as marked.

The picture presented by the original case was that of an acute febrile jaundice. There was a severe general infection which must have caused degenerative changes in the liver as well as in the kidney cells. The prolonged weakness and the general muscular pains during convalescence showed that the infection was a severe general one.

Experiments with a bacillus isolated from the urine, showed it to be toxic to guinea pigs and in a lesser degree to rabbits. Acute hepatitis was marked in all cases that came to autopsy. Acute nephritis was also found. In the animal which survived the innoculation, the urine showed bacteriuria with albumin and casts.

BIBLIOGRAPHY.

1.—Weil: *Deut. Arch. f. Klin. Med.*, Bd. XXXIX, 1886.
2.—Jäger: *Hygiene u. Infect. Krankheiten*, Bd. XII, 1892.
3.—H. H. Brooks: *Archives of Neurology and Psycho-path.*, Vol. III.
4.—E. Libman: Contrib. to Study of Weil's Disease, *Philad. Med. Journ.*, *1899*, p. 620.

Discussion.

Dr. Harlow Brooks said that the case which he had originally reported had followed very closely the course described by Dr. Satterlee. His case, however, had died, and at autopsy the changes in the liver had been found to be very extreme, almost to be compared with the changes in acute atrophy. He thought that the disease was very badly named, since the name would imply that the disease was a definite and specific one. Although there was a great similarity in the cases spoken

of by Dr. Satterlee by no means all cases showed the same bacteria. Dr. Brooks believed that almost any bacteria were capable of producing the changes. He had noticed that there were very few cases of Weil's disease reported in American literature, probably due to the fact that there were a great many cases of acute jaundice with albuminuria and presumably with bacteriuria which were not reported at all. He had himself recently seen a case which had followed the eating of large quantities of lobster salad. Such cases usually followed the eating of contaminated meats. The more recent work on the disease had tended to confirm the idea that it was not a specific disease and was not caused by a specific bacillus; but that it might be caused by a large group of bacteria. The cases were apt to have relapses. In regard to Dr. Satterlee's having been unable to reproduce the jaundice in the experimental animals, Dr. Brooks said that he had been himself unable to do so. He thought this might be due to the fact that jaundice was rare in the animals used for experiments. He had never seen it himself, nor had he ever seen it reported.

Dr. W. H. Park asked Dr. Satterlee exactly what connection he believed to exist between the bacteria, the lesions causing the acute jaundice and the bacteriuria. Did he think the bacteria which through their toxins excited the lesions in the region of the liver passed by the blood to the kidney; or had there not probably been either an increase in a previous latent infection of some part of the urinary tract or the starting up of an independent infection through the influence on the body resistance of the infection causing the jaundice?

Dr. Satterlee said that as far as could see there had been no previous disease of the kidneys. He could not say positively that there had not been bacteria there

before. He had examined the urine frequently and it had showed no signs of bacteriuria.

Dr. Park said that in this case the urine seemed to be a suitable soil for the growth of the bacteria found, since they continued present for many months after all symptoms had abated and might therefore have been present in small numbers before without having produced any noticeable changes in the urine.

A CASE OF BILHARZIA HÆMATOBIA.

EUGENE H. POOL, M.D.

Dr. Pool presented a case of Bilharzia hæmatobia; the interest of which, he said, was due, in the first place, to the infrequency with which the trouble was seen in this part of the world, and secondly, to the interesting phenomenon which might be noted under the microscope; namely, the development of the embryo from the ovum.

The patient was a young man, twenty years of age, an Algerian by birth, a sailor by occupation. When he was eight years of age he went to Ismailia on the Suez Canal, Egypt, and remained there for five years. During that time he went in swimming almost daily, both in the Suez itself, and in the neighboring streams and ponds. The next five years had been spent on a Greek ship in the Mediterranean. He had left this vessel two years ago at Marseilles, and there he contracted gonorrhœa four days after intercourse. He had had with that attack very little pain and frequency of micturition, but had had a characteristic discharge which disappeared at the end of fifteen days under urethral injections. A fortnight later he went back to Algiers where it was in-

Fig. I.—Embryo in Shell.

Fig. II.—Free swimming Embryo of Bilharzia.

tensely hot, and shortly after reaching the country, as he supposed on account of the intense heat, he noticed that all of his urine was diffusely red. He remained in Algiers for three or four months and the condition persisted. He had no other symptoms and was able to go about his regular occupations. As soon as he left the country the diffusely bloody urine changed to the characteristic hæmaturia of the disease; viz.: after he had passed a normal amount of a fairly normal looking amber colored urine and when the bladder was apparently empty, with an involuntary spasm there was discharged a dram or two of a thin bloody fluid. This condition had persisted up to the present time, intermittently. The man spent nine months in Cuba, and for the last six months had been in the United States. At the present time he also complained of indefinite pain in the loins and of a feeling of weakness which prevented him from doing any earnest work.

The physical examination showed a well nourished young man. Examination of heart, lungs, abdomen, rectum, etc., was negative.

The following was the report of the examination of urine:

April 8th. Volume voided in twenty-four hours, 1950 c.c. Reaction, acid. Color, amber. Sediment, moderate in amount, reddish white. Specific gravity, 1.019. Albumin, trace. Sugar, negative. Urea, 41.301 grams in 24 hours. Indican, no excess. Acetone, negative. Chloride, 13.6 grams in 24 hours. Phosphates, no excess. Examination of sediment obtained by centrifuge showed: blood, very small amount; pus, small amount; mucus, small amount; extremely few hyaline casts; numerous squamous epithelial cells; many ova of the Bilharzia; and numerous structureless threads.

Examination of blood: Red cells, 3,340,000. Hæmoglobin, 86%. Leucocytes, 6,600. Differential count: Small lymphocytes, 31.5%. Large lymphocytes, 6.5%. Polymorphonuclear neutrophiles, 52.5%. Eosinophiles, 9.5%. Dr. Pool also exhibited three photomicrographs; the first showed the ovum consisting of shell inclosing the embryo (Fig. 1); the second showed the egg shell from which the embryo had migrated; and the third, the embryo after its exit from the shell. (Fig. 2) Under the microscope were shown specimens of the patient's urine containing the ova; and also diluted urine in which the development of embryos from ova was in progress.

DIFFERENTIAL LEUCOCYTE COUNT IN THE EARLY DAYS OF TYPHOID FEVER.

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The present knowledge regarding the differential leucocyte count in typhoid fever has been chiefly obtained through the studies of Türck, Naegeli, Ouskow, Thayer and Winter. From their observations it may be considered as definitely settled that in uncomplicated cases of this disease, the polymorphonuclear neutrophilic leucocytes are relatively and absolutely decreased while the large mononuclears are always relatively and usually also absolutely increased in number. Furthermore the small mononuclears are relatively and absolutely decreased, normal or increased, the eosinophiles being both relatively and absolutely subnormal. The limits within

which these variations usually occur are, however, not accurately settled since the figures given by different authors vary widely; for example, the average relative reduction of polymorphonuclear neutrophilics as observed in ten cases by Winter is nearly 10% greater than that found by Thayer in a like number of cases,—the observations of both authors having been made with substantially the same technic and at the same period of the disease, namely during the second week. It is generally supposed and stated, moreover, that the peculiar relative variations in the number of leucocytes in typhoid fever are first manifest during the second week, from which time on they increase in intensity in greater or less degree. This supposition is mainly if not entirely based upon the observations of Thayer since of the observers above mentioned he is the only one who reports examinations previous to this time,—that is, during the first week of the disease. He reports as thus examined in the first week twelve cases and gives the following average figures for the percentages of the various leucocytes present;—small mononuclears, 12.9%; large mononuclears, 12.4%; polymorphonuclear neutrophilics, 74%; eosinophiles, .5%.

Curiously enough, in these cases Thayer has not stated the grounds upon which the diagnosis of typhoid fever was made, nor the facts which led him to suppose that the counts were, in truth, made during the first week of the disease. Furthermore, the counts were not made individually by him but by different internes at the Johns Hopkins Hospital. The number of leucocytes counted in each case varied. In some 200 was the number, while in others upwards of 300 were enumerated. I do not bring forward these facts with respect to Dr. Thayer's counts with any intent or desire of minimizing their value, which is great, or of drawing them under any

suspicion whatsoever; but simply to make a fair statement concerning the conditions under which they were obtained and to show that increased observations might demonstrate his conclusions were not universally applicable and to be applied to all cases of typhoid during the first week.

In view of all the circumstances, therefore, nearly two years ago I began a series of observations upon the differential leucocyte count in the early days of the disease, utilizing therefore cases as they from time to time appeared in my own practice or in those other physicians and which I had the opportunity of examining. I should not deem it worth while at the present time to communicate my results, but for the fact that they vary quite markedly from those found by Thayer during the first week, and have convinced me that in many instances the differential leucocyte count which may be called characteristic of typhoid is present, contrary to general supposition, during the first week of the disease.

Technic.

The blood drop was obtained in the ordinary manner without pressure.

The smears were made by the cover glass method and thinly spread. No thick smears in which the leucocytes were entangled in masses of red blood cells were used.

In staining the smears one cover glass from each patient was stained with Ehrlich's Triacid mixture and the other with either Jenner's or Wright's stain. The manipulations in using these stains were as follows:

Ehrlich's.—The smears were fixed by exposing them to dry heat for 2 minutes at 100°–120° C. They were then

covered with the stain and exposed thereto for 5 minutes. They were then washed off in distilled water, dried between filter paper and mounted in balsam.

Jenner's.—The smears were covered with the stain and exposed thereto for 2 minutes. During this time they were protected from the air by being placed in small double dishes. They were then washed *actively* in distilled water, dried in the open air and mounted in balsam.

Wright's.—The smears were covered with the stain and exposed thereto in the open air for 1 minute. Ordinary tap water was then added drop to drop to the stain upon the smear until there appeared upon the surface of the same a yellowish scum. The exposure was then continued for 3 minutes. They were then washed *actively* in tap water, dried between filter paper and mounted in balsam.

In making the counts, a $\frac{1}{12}$ inch oil immersion objective and a mechanical stage were used. One thousand leucocytes were counted with each stain; that is, in all, two thousand leucocytes from each patient. The count upon each cover glass was made without interruption until the full number of 1000 was counted. If interruption unavoidably occurred, the count was begun over again.

Differentiation of Leucocytes.

It is not necessary in this place to consider the differentiation of the leucocytes with the stains which were employed in this work, so far as the polymorphonuclear neutrophilics and eosinyphiles are concerned, since no difficulty attaches to the same with anyone who has had any experience in the examination of stained blood smears. It is a fact, however, that there is not between

different observers a precise uniformity concerning the differentiation of the small from the large mononuclears. For this reason I give the differentiation which has been observed in these studies between these two classes of leucocytes.

Ehrlich's.—Those mononuclear non-granular leucocytes which appeared in their entirety of a light blue or green color and were the same size or smaller than a red blood cell, were counted as small mononuclears and in addition all those mononuclear non-granular leucocytes which showed a nucleus of a light blue or green color surrounded by a narrow zone of buff colored protoplasm and which in their entirety were not larger than a red blood cell. All mononuclear non-granular leucocytes which were larger than a red blood cell were classified as large mononuclear. In most of these the nucleus was round and symmetrical in form, but in a few it was invaginated. The staining properties of the protoplasm of the large mononuclears varied. In the vast majority it appeared of a buff color, but in a *few* the protoplasm was poorly stained and was somewhat indistinct.

Jenner's.—Those mononuclear non-granular leucocytes which stained blue and were the size of a red blood cell or smaller were regarded as small mononuclear leucocytes. All other mononuclear non-granular leucocytes were counted as large mononuclears. Some of these showed a light blue nucleus surrounded by a narrow and more or less uneven rim of dark blue protoplasm.

Wright's.—Those mononuclear non-granular leucocytes whose nucleus was of a dark purplish blue color and whose protoplasm was a robin's egg blue, were counted as small mononuclears provided they were not distinctly larger than a red blood cell. If larger, they were classified as large mononuclears. In addition those cells were

regarded as large mononuclears which were non-granular and mononuclear, the nucleus being a blue or lilac and protoplasm pale blue or dark. As a rule these latter leucocytes were larger than a red blood cell, but in a few instances they were smaller; in such instances they were classified as small mononuclears.

Epitome of Cases.

The number of typhoid cases studied was sixteen. The diagnosis was established subsequent to the count as follows: in seven by the isolation of typhoid bacilli from the feces, using the Hiss method; in six by the presence of a positive Widal reaction (1–20 or above); and in three on a clinical basis by the appearance of a typical typhoid roseola, an enlarged spleen and a typical clinical course. In these three cases circumstances interfered with the carrying out of either a Widal or isolation test.

In studies of this nature after having established the diagnosis of the cases examined beyond the peradventure of a doubt, it becomes necessary to demonstrate with as much certainty as possible the fact that the examinations made were in reality conducted at the specified period of the disease to which claim is made. In a disease like typhoid fever such a demonstration becomes at once a matter of the greatest difficulty. When all the circumstances in connection with a given case are taken into consideration one may honestly feel that he is dealing with a specified period of the disease (such as in these cases the first week), and yet to prove that such is the case often becomes well nigh an impossibility. To my mind there is but one factor which can settle such a question beyond a reasonable doubt. I refer to the date of the first appearance of the eruption. Authorities differ concerning the limiting days of the disease within

which roseola may first appear. Comparatively few give definite figures. Most say that it usually first appears during the latter part of the first or the beginning of the second week and that it may precede or succeed this period by a few days. Osler, however, does give definite figures and says from the 7th to the 10th day. While there can be no doubt but that it *may* first appear a few days earlier or later than this period, I nevertheless believe that these dates may be regarded as practical working figures. In the endeavor to establish the date of my examinations I feel justified in accepting them, recognizing that they are not infallible, but believing that they constitute a standard from which conclusions may safely be drawn. Admitting that in a given series of cases the roseola first appeared on the latter of these days, (the 10th) if it can be shown that in each case a given examination was made at least three days previous to this it would appear beyond a reasonable doubt that such an examination was made within the first week of the disease. Of course this presupposes that each case was seen early and that frequent and careful examinations were made to detect the first appearance of the roseola. In all of these cases such frequent and careful examinations were made, and they were certainly all seen early in the course of the disease. Nine of the sixteen cases respond to this test—that is, the differential and total counts were made at least three days previous to the first appearance of the roseola. Here follows a detailed consideration of each of these nine cases:

Case I.—L. H. Age 25 years. Fairly characteristic prodromal history of typhoid fever. Leucocyte counts made on what was probably the third day. Evening temperature, 102.2° F. Pulse, 92. Characteristic typhoid roseola first appeared five days thereafter on

what was probably the eighth day. Widal reaction (1–20) first positive on what was probably the ninth day. Previous Widal tests had been made on the third and sixth days. No attempts were made to isolate typhoid bacilli from the feces or urine.

Leucocyte counts made on day above indicated:

Total count, - 5250;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclear,	22.1%	19.3%
Large "	12.4	15.7
Polymorphonuclear neutrophyilics,	62.3	62.0
Eosinophiles,	3.1	3.0
Basophilics (Mast Cells)	.1	
	100.0%	100.0%

Case II.—C. B. Age 15 years. Fairly characteristic prodromal history of typhoid fever. Leucocyte counts made on what was probably the third day. Evening temperature, 101.8° F. Pulse, 95. Characteristic typhoid roseola first appeared four days thereafter on what was probably the seventh day. No Widal tests were made. No attempts were made to isolate typhoid bacilli from feces or urine. Spleen first detected to be enlarged in the latter part of the second week. Subsequent clinical course typical of typhoid fever.

Leucocyte counts made on day above indicated:

Total count, - 7200;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclear,	21.2%	22.8%
Large "	19.4	18.2
Polymorphonuclear neutrophilics,	58.2	57.4
Eosinophiles,	1.0	1.6
Basophilics, (Mast Cells)	.2	
	100.0%	100.0%

CASE III.—A. C. Age 29 years. Indefinite prodromal history of typhoid fever. Leucocyte counts made on what was probably the fourth day. Evening temperature, 103° F. Pulse, 98. Characteristic typhoid roseola first appeared five days thereafter on what was probably the ninth day. Widal reaction (1–40) first positive on what was probably the fourteenth day. Previous Widal tests had been made on the fourth and eighth days. No attempts were made to isolate typhoid bacilli from feces or urine.

Leucocyte counts made on day above indicated:

Total count, - 5200;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	19.7%	20.4%
Large "	14.0	12.1
Polymorphonuclear neutrophilics,	65.2	65.5
Eosinophiles,	1.0	2.0
Basophilics (Mast Cells)	.1	
	100.0%	100.0%

CASE IV.—B. N. Age 21 years. Characteristic prodromal history of typhoid fever with epistaxis. Leucocyte counts made on what was probably the fourth day. Evening temperature, 100.1° F. Pulse, 93. Characteristic typhoid roseola first appeared four days thereafter on what was probably the eighth day. No Widal tests were made. No attempts were made to isolate typhoid bacilli from feces or urine. Enlargement of spleen first detected on the twelfth day. Subsequent clinical course characteristic of typhoid fever.

Leucocyte counts made on day above indicated:

Total count, - 6100;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	9.2%	12.8%
Large "	28.1	24.1
Polymorphonuclear neutrophilics,	59.9	61.7
Eosinophiles,	2 8	1.4
Basophilics (Mast Cells)	0.0	
	100.0%	100.0%

Case V.—A. H. Age 9 years. Characteristic prodromal history of typhoid fever. Leucocyte counts made on what was probably the seventh day. Evening temperature, 102° F. Pulse, 97. Characteristic typhoid roseola appeared three days thereafter on what was probably the tenth day. Widal reaction (1–20) first positive on the thirteenth day. A previous Widal test had been made on the seventh day. Typhoid bacilli were isolated from the feces by the Hiss method at the first attempt on the tenth day, and from the urine at the first attempt on the twenty-third day. Urine contained albumin but no casts.

Leucocyte counts made on day above indicated:

Total count, - 5000;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	11.6%	14.2%
Large "	24.2	22.0
Polymorphonuclear neutrophilics,	63.5	63.0
Eosinophiles	.7	.8
Basophilics (Mast Cells)	0.0	
	100.0%	100.0%

Case VI.—M. A. Age 26. No prodromal history. Onset sudden. Leucocyte counts made on what was probably the fourth day. Evening temperature, 101° F. Pulse, 85. Characteristic typhoid roseola first appeared four days thereafter on what was probably the eighth day. No Widal tests were made. No attempts were

made to isolate typhoid bacilli from feces or urine. Enlargement of spleen first detected on the seventeenth day. Subsequent clinical course was typical of typhoid fever.

Leucocyte counts made on day above indicated:

Total count, - 4750;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	19.0%	20.2%
Large "	28.4	29.3
Polymorphonuclear neutrophilics,	51.1	49.1
Eosinophiles,	1.4	1.4
Basophilics (Mast Cells)	.1	0.0
	100.0%	100.0%

Case VII.—J. E. Age 6 years. No prodromal history of typhoid fever. Leucocyte counts made on what was probably the third day. Evening temperature, 104° F. Pulse, 105. Characteristic typhoid roseola first appeared five days thereafter on what was probably the eighth day. Widal reaction (1–40) first positive on the twelfth day. Previous Widal tests had been made on the third and eighth days. Typhoid bacilli first isolated from the feces by the Hiss method on the fifteenth day. Similar isolation tests had been made on the eighth and twelfth days. No attempts were made to isolate typhoid bacilli from the urine.

Leucocyte counts made on day above indicated:

Total counts, - 9000;
Differential counts;

	Wright's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	21.2%	21.0%
Large "	26.3	26.9
Polymorphonuclear neutrophilics,	50.1	50.0
Eosinophiles,	2.4	2.1
Basophilics (Mast Cells)	0.0	
	100.0%	100.0%

Case VIII.—H. W. Age 11 years. Indefinite prodromal history of typhoid fever. Leucocyte counts made on what was probably the fourth day. Evening temperature, 102.5° F. Pulse, 100. Characteristic typhoid roseola first appeared five days thereafter on what was probably the tenth day. Widal reaction (1–40) first positive on the ninth day. A previous Widal test had been made on the fourth day. No attempts were made to isolate typhoid bacilli from the feces or urine.

Leucocyte counts made on day above indicated:

Total count, - 6000;
Differential count;

	Wright's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	14.2%	14.1%
Large "	23.3	23.1
Polymorphonuclear neutrophilics,	60.0	60.4
Eosinophiles,	2.2	2.4
Basophilics (Mast Cells),	.3	
	100.0%	100.0%

Case IX.—N. H. Age 15 years. Fairly characteristic prodromal history of typhoid fever. Leucocyte counts made on what was probably the sixth day. Evening temperature, 103.6° F. Pulse, 106. Characteristic typhoid roseola first appeared three days thereafter on what was probably the ninth day. Widal reaction (1–40) first positive on the fifteenth day. Previous Widal tests had been made on the sixth and tenth days. No attempts were made to isolate typhoid bacilli from feces or urine.

Leucocyte counts on day above indicated:

Total count, - 5500;
Differential count;

	Wright's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	15.2%	15.8%
Large "	18.6	18.7
Polymorphonuclear neutrophilics,	64.1	64.5
Eosinophiles,	2.0	1.0
Basophilics (Mast Cells),	.1	
	100.0%	100.0%

These nine cases show the following average percentages for the various leucocytes present. In computing the averages, I have utilized the counts made with Ehrlich's triacid stain. The averages from those made with Jenner's and Wright's stains show only a variance of a fraction of 1% from those computed from smears stained with Ehrlich's mixture:

Small Mononuclears.	*Large Mononuclears.*	*Polymorphonuclear neutrophilics.*	*Eosinophiles.*
17.8%	21.1%	59.4%	1.7%

The lowest and highest percentages for the various leucocytes are as follows:

	Small Mononuclears.	*Large Mononuclears.*	*Polymorphonuclear neutrophilics.*	*Eosinophiles.*
Lowest,	12.8%	12.1%	491.%	.8%
Highest,	22.8	29.3	65.5	3.0

The remaining seven of the sixteen cases from a consideration of the history and the clinical symptoms I believe to have been within the first week when the leucocyte counts were made. I cannot, however, demonstrate beyond a reasonable doubt such was the case. I hence include them in this report, but have not used them in computing the averages. The following is a detailed consideration of them.

CASE I.—M. A. Age, 18 years. Typical prodromal history of typhoid fever with epistaxis. Leucocyte counts were made on what was probably the sixth day. Evening

temperature, 103.1° F. Pulse, 101. No characteristic typhoid roseola was ever observed. Widal reaction (1–20) first positive on what was probably the eighth day. A previous Widal test had been made on what was probably the sixth day. Typhoid bacilli were first isolated from the feces by the Hiss method on what was probably the twelfth day. Typhoid bacilli were never isolated from the urine, although attemps were made by the same method on the twenty-first, thirtieth and forty-first days.

Leucocyte counts made on day above indicated:

Total count, - 4850;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	14.5%	16.2%
Large "	21.1	18.1
Polymorphonuclear neutrophilics,	62.3	63.5
Eosinophiles,	2.0	2.2
Basophilics (Mast Cells),	.1	
	100.0%	100.0%

Case II.—A. W. Age, 27 years. No prodromal history. Leucocyte counts made on what was probably the third day. Evening temperature, 103.4° F. Pulse, 109. No characteristic typhoid roseola was ever observed. Widal reaction (1–40) first positive on what was probably the tenth day. A previous Widal test had been made on the third day. No typhoid bacilli were ever isolated from the feces although attempts were made by the Hiss method on what were probably the third, tenth, twelfth, eighteenth, twenty-sixth and thirty-fourth days. No typhoid bacilli were ever isolated from the urine, although attempts were made by the same method on the twenty-sixth and thirty-fourth days. The case ran a characteristic typhoid clinical course with an enlarged spleen and the Widal reaction (1–40) remained positive into convalescence.

Leucocyte counts made on day above indicated:

Total count, - 5700;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	21.1%	20.0%
Large "	25.2	23.9
Polymorphonuclear neutrophilics,	52.7	54.1
Eosinophiles,	1.0	2 0
Basophilics (Mast Cells),	0.0	
	100.0%	100.0%

CASE III.—E. M. Age 22 years. Characteristic prodromal history of typhoid fever. Leucocyte counts made on what was probably the fifth clinical day. Evenning temperature, 102.2° F. Pulse, 100. Characteristic typhoid roseola first appeared two days thereafter on what was probably the seventh day. Widal reaction (1-20) first positive on the twelfth day. Previous Widal tests had been made on the fifth and seventh days. Typhoid bacilli were isolated from the feces by the Hiss method on the tenth day. Typhoid bacilli were never isolated from the urine though attempts were made by the same method on the twenty-eighth, thirtieth, thirty-second and thirty-eighth days.

Leucocyte counts made on day above indicated:

Total count, - 4100;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	8.2%	9.1%
Large "	32.4	30.1
Polymorphonuclear neutrophilics,	57.6	59.2
Eosinophiles,	1.7	1.6
Basophilics (Mast Cells),	.1	
	100.0%	100 0%

CASE IV.—D. P. Age 7 years. Fairly characteristic prodromal typhoid history. Leucocyte counts made on

what was probably the fifth day. Evening temperature, 101.3° F. Pulse, 108. No characteristic typhoid roseola was ever observed. Typhoid bacilli first isolated from the feces on what was probably the sixth day and from the urine on the thirty-second day. Widal reaction first positive (1–50) on what was probably the eighth day. A previous Widal test had been made on the fifth day. A complicating measles developed on what was probably the ninth day. Total leucocyte count on this day, 4500. A differential leucocyte count on this day gave the following figures:

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	9.8%	8.9%
Large "	3.2	3.1
Polymorphonuclear neutrophilics,	86.1	86.9
Eosinophiles,	.9	1.1
Basophilics (Mast Cells),	0.0	
	100.0%	100.0%

Leucocyte counts on day above indicated (the fifth):

Total count, - 6750;
Differential count;

	Jenner's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	19.8%	19.9%
Large "	25.1	25.7
Polymorphonuclear neutrophilics,	54.5	53.7
Eosinophiles,	.5	.7
Basophilics (Mast Cells),	0.0	
	100.0%	100.0%

Case V.—F. A. Age 30 years. Indefinite prodromal typhoid history. Leucocyte counts made on what was probably the fifth day. Evening temperature, 102.9° F. Pulse, 100. Characteristic typhoid roseola first appeared two days thereafter on what was probably the seventh day. Widal reaction (1–40) first positive on

what was probably the twelfth day. A previous Widal test had been made on the fifth day. No attempts were made to isolate typhoid bacilli from the feces. Typhoid bacilli were isolated from the urine by the Hiss method at the first attempt on the thirtieth day.

Leucocyte counts made on the day above indicated:

Total count, - 4000;
Differential count;

	Wright's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	16.2%	16.7%
Large "	31.1	30.2
Polymorphonuclear neutrophilics,	50.1	52.1
Eosinophiles,	2.3	1.0
Basophilics (Mast Cells),	.3	
	100.0%	100.0%

Case VI.—M. M. Age 21, years. Indefinite prodromal typhoid history. Leucocyte counts made on what was probably the fifth day. Evening temperature, 103.5° F. Pulse, 112. No characteristic typhoid roseola was ever observed. Widal reaction (1–40) first positive on what was probably the fourteenth day. Previous Widal tests had been made on the fifth and eleventh days. No attempts were made to isolate typhoid bacilli from the feces or urine. Subsequent clinical course was typical of typhoid fever.

Leucocyte counts made on day above indicated:

Total count, - 7000;
Differential count;

	Wright's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	10.1%	10.2%
Large "	24.9	24.7
Polymorphonuclear neutrophilics,	63.9	64.0
Eosinophiles,	1.0	1.1
Basophilics, (Mast Cells),	.1	
	100.0%	100.0%

Case VII.—E. S. Age, 14 years. Fairly characteristic prodromal history of typhoid fever. Leucocyte counts made on what was probably the fifth day. Evening temperature 102.4° F. Pulse, 100. Characteristic typhoid roseola first appeared two days thereafter on what was probably the seventh day. Widal reaction (1–20) first positive on the same day. A previous Widal test had been made on the fifth day. Typhoid bacilli were isolated from the feces by the Hiss method at the first attempt on what was probably the eighth day. No attempts were made to isolate typhoid bacilli from the urine.

Leucocyte counts made on day above indicated:

Total count, - 5750;
Differential count;

	Wright's Stain.	*Ehrlich's Triacid Stain.*
Small mononuclears,	19.0%	19.2%
Large "	18.9	18.4
Polymorphonuclear neutrophilics,	60.1	60.5
Eosinophiles,	1.9	1.9
Basophilics (Mast Cells),	.1	
	100.0%	100.0%

If the average figures for the various leucocytes in these seven cases are computed it will be found that they do not materially differ from the averages obtained in the nine cases which precede them.

Summary.

By comparing the figures found in the nine cases above referred to with those of Thayer in the first week, it will be at once seen that the relative number of polymorphonuclear neutrophilics is in the average much lower (14.6%) while that of the large mononuclears is much higher (8.7%). The small mononuclears and

eosinophiles also show somewhat higher figures, but the marked decrease in the polymorphonulear neutrophilics is principally due to the marked increase in large mononuclears. My figures more nearly correspond so far as the relative number of polymorphonuclear neutrophilics and eosinophiles is concerned, to those given by Winter as found by him in ten cases during the second week which are as follows:

Small Mononuclears	*Large Mononuclears.*	*Polymorphonuclear neutrophilics.*	*Eosinophiles.*
5.8%	31.5%	61.4%	1.3%

In endeavoring to account for the marked variance between my figures and those of Thayer, it might be supposed that I had been in error in my differentiation between the large mononuclears and polymorphonuclear neutrophilics and had counted many cells among the former when in reality they should have been classified among the latter. This thought might carry great weight provided these observations had been made upon the blood of a disease like diphtheria which we know from the valuable observations of Ewing in 1895 shows the presence of a large number of leucocytes which possess deficient staining properties, so that in the polymorphonuclear neutrophilics the neutrophilics granules are poorly stained and frequently almost entirely absent, while the protoplasm of the large mononuclears also stains faintly. It might be supposed that in examining stained smears of such a blood, the presence of such leucocytes would cause confusion to a man not familiar with Ewing's work, and lead him to misclassify large numbers of polymorphonuclear neutrophilics and large mononuclears. Now as a matter of fact, the blood of typhoid fever shows the presence of few if any such deficiently staining leucocytes and all who have busied themselves

with this line of work are agreed that the increase in large mononuclears which is apparent to all during the middle and latter part of the disease, is principally if not entirely due to an increase in typical large mononuclear forms which could not be misclassified by anyone who has had any experience in the examination of stained blood smears. I do not think, therefore, that in these observations I have made the error of classifying any polymorphonuclear neutrophilics among the large mononuclears. The only other supposition which could reasonably be advanced to account for the variance in these counts is that in reality my counts were made later in the disease than I claim. This point I have covered in a previous portion of this article and I believe I have demonstrated so far as it is possible that my counts were made during the first week.

If my opinion be correct, the value of the differential in combination with the total leucocyte count in the early diagnosis of the disease, becomes a matter for serious consideration. I do not propose to enter into more than a preliminary consideration of this phase of the subject, because of the limited number of my observations. The acute diseases which usually show a normal total leucocyte count or a total hypoleucocytosis and which so far as the total leucocyte count is concerned would have to be differentiated from typhoid, are measles, influenza, pure tubercular infection and malaria. From a practical standpoint, however, only the latter two of these deserve consideration since measles and influenza can easily be excluded upon a clinical basis. With the differential leucocyte count in pure tubercular infection I have had no personal experience. So far as I am able to ascertain, there is but one case in literature. This is a case of acute miliary tuberculosis and is cited by Cabot

in the last edition of his work. Here the relative number of polymorphonuclear neutrophilics was 91.64%. With the differential leucocyte count in malaria I have had a small experience (seventeen cases). The following are the total and differential counts found. In all of the cases, malarial organisms were abundantly present in the blood. The number of leucocytes counted was 1000.

Case I. Tertian. Total count, 4,500.
Differential count;

	Jenner's Stain.
Small mononuclears,	13.2 per cent.
Large "	32.4 "
Polymorphonuclear neutrophilics,	54.0 "
Eosinophiles,	.4 "
Basophilics (Mast Cells),	.0 "
	100.0 per cent.

Case II. Tertian. Total count, 16,000.
Differential count;

	Goldhorn's Stain.
Small mononuclears,	1.6 per cent.
Large "	14.4 "
Polymorphonuclear neutrophilics,	84.0 "
Eosinophiles,	0.0 "
	100.0 per cent.

Case III. Tertian. Total count, 12,000
Differential count;

	Goldhorn's Stain.
Small mononuclears,	1.4 per cent.
Large "	21.6 "
Polymorphonuclear neutrophilics,	77.0 "
Eosinophiles,	0.0 "
	100.0 per cent.

Case IV. Tertian. Total count, 5,000.
Differential count;

	Goldhorn's Stain.
Small mononuclears,	5.0 per cent.
Large "	18.1 "
Polymorphonuclear neutrophilics,	76.9 "
Eosinophiles,	0.0 "
	100.0 per cent.

Case V. Tertian. Total count, 7,000.
Differential count;

	Wright's Stain.
Small mononuclear,	32.8 per cent.
Large "	42.0 "
Polymorphonuclear neutrophyilics,	25.0 "
Eosinophiles,	.2 "
	100.0 per cent.

Case VI. Tertian. Total count, 6,000.
Differential count;

	Ehrlich's Triacid Stain.
Small mononuclear,	32.4 per cent.
Large "	19.0 "
Polymorphonuclear neutrophilics,	47.2 "
Eosinophiles,	1.4 "
	100.0 per cent.

(A blood smear made from the same patient at the same time and stained with Wright's stain showed a large number of malarial organisms.)

Case VII. Tertian. Total count, 7,000.
Differential count;

	Wright's Stain.
Small mononuclears,	6.2 per cent.
Large "	9.2 "
Polymorphonuclear neutrophilics,	84 4 "
Eosinophiles,	0.0 "
Basophilics (Mast Cells),	.2 "
	100.0 per cent.

Case VIII. Tertian. Total count, 4,000.
Differential count;

	Ehrlich's Triacid Stain.
Small mononuclears,	17.0 per cent.
Large "	22.0 "
Polymorphonuclear neutrophilics,	60.5 "
Eosinophiles,	.5 "
	100.0 per cent.

(A blood smear made from the same patient and at the same time and stained with Wright's stain, showed the abundant presence of malarial organisms.)

CASE IX. Tertian. Total count, 4,000.
Differential count;

	Wright's Stain.
Small mononuclears,	4.0 per cent.
Large "	49.0 "
Polymorphonuclear neutrophilics,	47.0 "
Eosinophiles,	0.0 "
	100.0 per cent.

CASE X. Tertian. Total count, 4,500.
Differential count;

	Wright's Stain.
Small mononuclears,	2.0 per cent.
Large "	35.0 "
Polymorphonuclear neutrophilics,	61.5 "
Eosinophiles,	1.5 "
	100.0 per cent.

CASE XI. Tertian. Total count, 11,000.
Differential count ;

	Wright's Stain.
Small mononuclears,	6.8 per cent.
Large "	37.8 "
Polymorphonuclear neutrophilics,	52.2 "
Eosinophiles	3.2 "
	100.0 per cent.

CASE XII. Aestivo-Autumnal. Total count, 4,000.
Differential count;

	Wright's Stain.
Small mononuclears,	3.0 per cent.
Large "	43.0 "
Polymorphonuclear neutrophilics,	52.5 "
Eosinophiles,	1.5 "
	100.0 per cent.

CASE XIII. Aestivo-Autumnal. Total count, 11,000.
Differential counts;

	Wright's Stain.
Small mononuclears,	10.0 per cent.
Large "	15.8 "
Polymorphonuclear neutrophilics,	74.2 "
Eosinophiles,	0.0 "
	100.0 per cent.

Case XIV. Aestivo-Autumnal. Total count, 6,500.
Differential count;

	Wright's Stain.
Small mononuclears,	11.4 per cent.
Large "	12.5 "
Polymorphonuclear neutrophilics,	75.3 "
Eosinophiles,	.8 "
	100.0 per cent.

Case XV. Aestivo-Autumnal. Total count, 7,000.
Differential count;

	Wright's Stain.
Small mononuclears,	10.1 per cent.
Large "	9.5 "
Polymorphonuclear neutrophilics,	80.0 "
Eosinophiles,	.4 "
	100.0 per cent.

Case XVI. Aestivo-Autumnal. Total count, 5,000.
Differential count;

	Wright's Stain.
Small mononuclears,	8.6 per cent.
Large "	15.4 "
Polymorphonuclear neutrophilics,	75.1 "
Eosinophiles,	.9 "
	100.0 per cent.

Case XVII. Quartan. Total count, 6,000.
Differential count;

	Wood's Stain.
Small mononuclears,	6.5 per cent.
Large "	24.1 "
Polymorphonuclear neutrophilics,	69.3 "
Eosinophiles,	.1 "
	100.0 per cent.

From a consideration of the differential leucocyte counts present in these cases of malaria, it is at once seen that in many instances of this disease the differential count so closely simulates that present in many cases of typhoid during the first week that a separation is impossible. Certain of these cases of malaria (8 out of 17) show a marked relative increase in the polymorphonuclear neutrophilics and hence could be easily differen-

tiated from typhoid. By looking over them it will be seen that as a rule they show a much less relative number of eosinophiles than do the typhoid cases. These cases of malaria were all febrile cases and the smears were made at the time they appeared for diagnosis. In them the relative number of eosinophiles was almost always below 1.5 per cent and usually below 1 per cent, while the reverse holds true for the typhoid counts. Just how important a point this is in separating the differential counts of these diseases, I do not know. This point can only be determined by increased observations.

Conclusions.

1. Many cases of typhoid fever contrary to general supposition show the characteristic differential leucocyte count within the first week of the disease.

2. The definite value of the differential leucocyte count in the early diagnosis of typhoid fever is as yet problematical; but it seems certain that when used in addition to the total leucocyte count, more information may be obtained than by employing the total count alone.

In conclusion it gives me pleasure to acknowledge my indebtedness to Prof. T. Mitchell Prudden and Dr. Francis C. Wood for valuable suggestions during this work.

REFERENCES:

Türck: *Klinische Blutuntersuchungen*, 1898.

Ouskow: Cited by Türck.

Nagaeli: *Deutsches Archiv für klinische Medizin*, 1900. Bd. XXIII, page 93, and Bd. LXVII, page 279.

Winter: *Dublin Journal Medical Sciences*, Oct. 1901.

Thayer: *John Hopkins' Hospital Reports*, 1900. Vol. VIII, Nos. 3 to 9.

Ewing: *New York Medical Journal*, 1895.

Cabot: Clinical Examination of the Blood.

A SIMPLE METHOD OF OBTAINING BLOOD FROM THE RABBIT.

AUGUSTUS WADSWORTH, M.D.

Dr. Wadsworth demonstrated a simple method which he used for obtaining blood from the rabbit. He said that although he had usually had little difficulty in bleeding rabbits, there were animals, especially those anaemic or emaciated, which bled with great difficulty from the ear veins, yielding only a few drops at the most. His experience in bleeding rabbits had brought out the fact that there was less difficulty in summer than in winter, and on warm than on cold days, and when the animal was tilted head downwards. He had therefore adopted the method of warming and tilting his animals, which brought the blood into the peripheral circulation and distended the ear veins. Anaemic and emaciated animals which had proved refractory yielded under these conditions sufficient blood (five c.c. or more) for most purposes. After having tried various methods of warming his animals, Dr. Wadsworth had finally adopted the plan of putting them on a hot water bed. An ordinary commercial rubber bag, sixteen inches square, was partially filled with hot water and placed cornerwise on an animal tray. The animal, placed on the bag, was tied by its legs to the angles of the tray, and a blanket, wrapped around the bag and animal, increased the surface in contact with the water bag and kept in the heat. The rabbit was then ready to be tilted. A board longer than the tray, with a support behind by means of which the angle might be varied, and in front two right angle hooks screwed about six inches from the bottom of the board to hold the tray, served this purpose very well. The ear, previously shaved and sterilized with carbolic

solution and alcohol, hung down below the tray and a longitudinal incision was easily made with a sharp knife in the distended marginal vain. A transverse incision was to be avoided as it often allowed the vain to retract in its sheath and might sever the artary which accompanies the vein. A dull knife or a clumsy incision was apt to injure the walls of the vessels, so that clotting took place in the lumen of the vessels, much more quickly then if a clean incision were made. With this method twenty, or even more, c.c. of blood might easily be drawn at one time.

Discussion.

Dr. James Ewing asked Dr. Wadsworth whether he had tried this method on guinea-pigs.

Dr. Wadsworth said he had not.

A CONVENIENT METHOD FOR GROWING AND STORING VIRULENT PNEUMOCOCCI.

AUGUSTUS WADSWORTH, M.D.

Dr. Wadsworth called attention to the difficulty of keeping pneumococci alive and virulent for any length of time by ordinary culture methods. Any method which would do this would obviously be of great service to any one conducting a long series of experiments with this organism. Other observers had suggested various special media with which this might be accomplished.

Washbourn and Eyre had used agar smeared with rabbit's blood and had found their organism alive after some months. Although Dr. Wadsworth had not made an extended study as to how long the pneumococcus might

in exceptional instances remain alive and virulent under these conditions, he had frequently found such tubes sterile after a few weeks and therefore could not rely on this method. Bezancon and Griffon had suggested the serum of young rabbits as a favorable medium for growing and raising the virulence of the pneumococcus, for the preservation of living cultures they had recommended the serum of older rabbits. Guarniari had suggested a nutrient peptone meat infusion medium containing 4% gelatin and .3% to .4% agar; but the virulence was maintained for only five to fourteen days and the cultures were not viable for much longer periods.

Dr. Wadsworth then described a medium which in his experience had given more certain and better results than any he had as yet tried. He had chanced, while sterilizing ascitic or pleuritic sera at low temperatures (60° to 70° C.), to obtain in one instance a soft jelly-like consistency of the serum, which still retained its original transparency. Being unable to obtain this a second time with other sera, he then tried adding small quantities of agar (.3%) to the ordinary fluid serum, and found this made a very good substitute for the transparent semi-fluid serum. The serum to be used for this medium was drawn by sterile apparatus into sterile flasks and tubed as soon as possible. This was done to avoid the usual intermittent sterilization at low temperatures which often clouded the serum and could not be relied upon to destroy the spores of some contaminating organisms, the spores often failing to develop into vegetative forms in the undiluted serum. A dilution of the tubed serum with ordinary nutrient peptone agar in the proportion of about one part of agar to five of serum gave a semi-fluid medium which served all practical purposes. Dr. Wadsworth said it was well to avoid using media containing glucose

or other sugar which might be fermented by the pneumococcus, as rapid death and degeneration of the cells took place in such media. He had found cultures of pneumococcus in glucose broth dead after three days' incubation.

Compared with the ordinary agar or serum agar tubes the advantages were obvious and numerous:—there was practically no environmental change from that found in the body fluids of the animal; the growth was less compact; the products diffused more rapidly; and finally, subcultures were easily made with the platinum loop or with capillary pipettes by means of which colonies may be transferred from any part of the tube to fresh media.

In the semi-fluid serum the pneumococcus always grew luxuriantly along the puncture, forming colonies of considerable size, if allowed to reach its maximum growth. Microscopical preparations from such cultures showed less degeneration of the cells than in ordinary media. Furthermore, capsules were formed and were readily demonstrated by the use of gentian violet and washing with 20% copper sulphate, as recommended by Hiss. (*Centralblatt f. Bakt., 1902*, Bd. XXXI, p. 302.) Cultures incubated for twenty-four hours or less and placed in the ice box at from 8° to 10° C., had been found to be alive and virulent for eight or more months in the pure serum medium, and three or more months in the serum agar substitue medium. The virulence of the pneumococcus had not been accurately determined but the results of animal inoculations had been compared at an interval of three months in the case of the growth in the serum agar tubes and had failed to show any appreciable loss of virulence in 1 c.c. doses. Peptone broth subcultures from an agar serum tube stored for three months in the ice box, in doses of 1 c.c. had killed by

intrathoracic inoculations a medium sized rabbit in about eighteen hours with the usual local and general lesions of pneumococcus infection.

In view of the recent researches on bacteriolysis, and more particularly those of Walker on the increase in virulence of micro-organisms when grown in the sera of specifically immunized animals, such a medium as he had shown, Dr. Wadsworth thought, might become of considerable practical service if only the blood serum of the immunized animal be substituted for the ascitic or pleuritic fluids he had used. The organisms might thus be kept in the sera of any animal for which it had become adapted for a number of weeks with but little biological change.

Discussion.

Dr. W. H. Park asked Dr. Wadsworth whether he thought that the bacteria would live longer in a medium so prepared than they would if it contained more agar. He had himself used .7% agar.

Dr. Wadsworth said he thought that the advantages of having the medium as fluid as possible were that one could get at the colonies more easily and that it allowed growth to spread out into the medium. On pure theory he thought that if the products of the organisms were allowed to diffuse through the media, the organisms might perhaps retain their viability and virulence longer than on solid media; but he did not know what the results of experiments would show in regard to this. He thought the pure serum was preferable to the agar serum. In other tubes of pure unheated fluid serum which were kept some time and had evaporated to a semi-fluid consistency, the pneumococcus apparently remained alive and virulent for a considerable time as in the other tubes, though accurate observations had not

been recorded. He did not know just how long the virulence might be sustained in these tubes. He suggested that the less the serum was diluted the better.

Dr. N. E. Ditman read a preliminary report on the Quantitative Estimation of Albumin in the Urine, especially with regard to the method of Wassilyew. The paper will be published in detail later.

Dr. F. C. Wood said that he thought the method which Dr. Ditman had just described promised to be an excellent one. Wassilyew's paper had been practically buried in the Russian journals and the method had never been much used. The whole technique, however, was so simple and the results so accurate that he thought it an excellent clinical method for the determination of albumin. No care was required in neutralizing the urine and in two or three minutes a determination could be obtained. Dr. Ditman would pursue the matter further, especially with the urines containing higher albumoses in order to see how much these albumoses influence the reactions, the tabulated results showing only globulins and albumins.

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DR. WILLIAM H. PARK, *President.*

THE DYSENTERY BACILLUS IN A SERIES OF CASES OF INFANTILE DIARRHOEA.

MARTHA WOLLSTEIN, M.D.,

Fellow of the Rockefeller Institute for Medical Research.

The systematic bacteriological study of the stools in cases of diarrhoea in infants and young children, following the observations of Duval and Bassett (1), was begun in October, 1902, with the view of determining the presence or absence of the Bacillus dysenteriae (Shiga) in all cases of diarrhoea, and whether any special group of clinical symtoms characterizes the cases in which the organism is found.

The material at the New York Foundling Asylum

(1) *American Medicine.* September 13, 1902.

having been placed at the disposal of the Rockefeller Institute, through the courtesy of the Medical Board, the cases sent to the ward for gastrointestinal diseases were examined as they came, without selection as to previous condition or disease. The occasion to study cases occurring during the winter months was considered especially interesting, as helping to establish the identity of this class of infantile diarrhoeas with the more frequent cases occurring during the summer months.

One hundred and fourteen cases were studied, of which one occurred at the New York Infant Asylum, three at the Nursery and Child's Hospital, fifty at the Babies' Hospital and sixty at the Foundling Asylum.

The technique used was that recommended by Dr. Flexner and his pupils. The stools were obtained as fresh as possible; where blood and mucus were present some was carefully taken on a sterile swab or platinum loop and suspended in neutral broth or peptone water. From this suspension agar plates were poured at once or within an hour. Duval found that the dysentery bacillus grows more profusely on agar that is slightly acid in reaction and throughout this work such a medium was used. After the plates had been in the thermostat for twenty-four hours a number of glucose agar tubes were inoculated from them, and twenty-four hours later the gas producing organisms were rejected. From the tubes without gas transplants were made to plain acid agar, and then the bacilli were tested as to their reaction with serum from a horse immunized with the Flexner Manila organism. This serum was obtained first from Dr. Park and later from Dr. Flexner. Finally, the two varieties of anti-dysenteric serum sent by Dr. Flexner to the Babies' Hospital, for the treatment of dysentery patients, were used. One of these is the "Harris", the other the

"Shiga" serum. Bacilli giving a positive reaction in a dilution of 1–50 were tested with both sera in dilutions up to 1–3000.

Stools from healthy infants were examined in ten cases. The dysentery bacillus was not found. Ten newly born infants were studied at the Lying-In Hospital; the stools being examined repeatedly from the age of twelve hours to eight days. In no instance was the dysentery bacillus present.

Conversely, no case from which the stools contained blood as well as mucus failed to show the dysentery bacillus in culture; but many of the negative cases contained large quantities of mucus and undigested food, without blood. It would seem that the tendency in cases of infection with the Bacillus dysenteriae is toward the classical picture of dysentery as regards the clinical symptoms.

Of the one hundred and fourteen cases studied, the dysentery bacillus was found in thirty-nine. The ages of the children varied from five weeks to four years; but only twenty-one were over one year old, and seven over two. The ages of the thirty-nine positive cases were as follows:—

2	months	3
3	"	3
5	"	3
6	"	9
7	"	2
8	"	4
10	"	2
11	"	1
12	"	3
13	"	1
15	"	2
18	"	1
21	"	1
2	years	3
3	"	1——39

Thus, twenty-seven were under one year old, and nine were under six months; while eight were between one and two years old, and four over two. Of these positive cases, twenty-eight died; two are still under observation; one left the hospital improved, but not well; and eight were cured.

The character of the stools varied greatly. Blood appeared in twenty, usually as small specks mixed with or streaking the mucus, and not in every stool. In three cases fluid blood was present, and many stools consisted entirely of blood and mucus. In every case mucus was passed, usually in very large amounts; curds and undigested food were also frequent. The stools varied from two to nine in twenty-four hours; were in most cases green in color; and accompanied by marked tenesmus in five instances. The absence of this symptom in a large number of cases is due, in part at least, to lack of observation. In uncomplicated cases the temperature ranged from 98° to 103° F., there being an initial rise; but in nine cases it never rose above 100.8° F. The fatal cases ran their course in three days to six weeks; by far the greater number dying during the first or early in the second week. Only three lived four weeks or more. Recovery took place after one to four weeks.

The bacillus isolated from these cases is a short, non-motile rod with rounded ends, sometimes almost coccoid in form, occurring singly or in pairs. It is Gram negative, and does not liquefy gelatine nor coagulate milk. No gas is formed in glucose, saccharose, nor lactose media. On agar plates the superficial colonies are pearly white,—characteristically so. Microscopically they are round, finely granular, and have regular edges which become irregular after forty-eight hours. The deep colonies are round or lenticular, also granular and

at first regular. Agar slants are dry, irregular at the edges, cream white in color, and spread but little beyond the line of inoculation. Neutral broth becomes cloudy, and a fine granular precipitate forms. Two of my cultures form a thin pellicle after five to six days, comparable to that described by Hiss (1) in the case of the Bacillus "Y".

No pellicle is developed by any of the other dysentery bacilli isolated in this series. Indol is formed in dextrose-free-broth after three days. In some individuals seven days elapse before the reaction appears. One bacillus (S.A.) isolated from the second case studied at the Foundling Asylum, forms no indol at any time. This culture is the only one of the thirty-nine in my series which does not ferment mannite, maltose and raffinose. In this and in its serum reactions it agrees with the Shiga and New Haven (Flexner) cultures used for control, and for which I am indebted to Dr. Park (2). All the rest form acid in the presence of mannite, raffinose and maltose, as well as levulose, galactose and glucose.

The agglutination reactions are fairly uniform. All the bacilli react with the Flexner Manila ("Harris") serum in dilutions of 1–50 to 1–3000 and with the Shiga serum up to 1–200 only. The culture S.A. which does not ferment mannite, reacts with the Shiga horse serum in dilutions of 1–3000, as do the Shiga and New Haven cultures. With the "Harris" serum it reacts in 1–500, as also do the Shiga and New Haven (Flexner) bacilli.

(1) Hiss: Transactions of the New York Pathological Society, Jan. 1903. *Medical News*, 1903, p. 289.

(2) The case from which this organism came was a boy of five months, whose stools consisted entirely of blood and mucus. Tenesmus was marked. He died on the third day, his blood having given a negative reaction a few hours before death. He had never been outside of New York City.

The blood reactions in young infants are, at first sight, uncertain in their appearance. On closer study they are seen to agree with the observations made by others in adult cases. Thus they were absent altogether in twelve cases; in six others the blood could not be obtained. In the remaining twenty-one a good reaction was present as follows:—

On the	1st	day in	1 case
"	3rd	"	1 "
"	4th	"	2 cases
"	6th	"	2 "
"	7th	"	3 "
"	8th	"	3 "
"	9th	"	1 case
"	13th	"	1 "

In seven cases the reaction was present only after the injection of antidysenteric sera; the serum having been given on the third, fourth, fifth, sixth, (two cases) eighth, and fourteenth days, and the blood having been found negative before that time.

Rosenthal (1) testing the agglutinations in thirty adult dysentery patients in Moscow, found the reaction absent during the first week, strong from the tenth to the twelfth days, and diminishing during the fourth and fifth weeks, the fifty-second day being the latest time at which he found it. In several fatal cases it was weak or entirely absent. Leonard Rogers (2) also found it present from the sixth day on, but least marked under ten days. Of the twelve positive cases which gave no serum reaction in my series, two died on the third day, one each on the fourth, fifth, and sixth days, two on the seventh day, and two on the twelfth; one mild case

(1) Rosenthal: *Deutsche Med. Wochenschr.* February 5, 1903.

(2) Rogers, *The Indian Medical Gazette.* February 1903.

recovered in one week and one case died after two weeks. So that of the twelve positive dysentery cases which gave no serum reaction, eight lasted one week and three were over with in two weeks.

The reactions were made with the bacillus obtained from the case, with that isolated from the first case examined (F. M.), and with the stock "Harris" and "Shiga" cultures. No reaction was obtained with the blood of any case and the Shiga organism. One hour was the time limit used.

Within twenty-four hours after an injection of "Harris" (Flexner Manila) serum, an excellent reaction was obtained in five cases, in dilution of 1–50 and 1–100. Eight days later the reaction was good in two cases, in 1–500 with the "Harris" bacillus; negative at all times with Shiga. In one instance the reaction was complete in two hours on the twenty-seventh day after the serum had been administered, when the child was discharged. Another child left the hospital twenty days after the inoculation, her blood giving a positive reaction in 1–100. Neither case had given a positive blood reaction before the administration of the serum, fourteen and eight days respectively after the onset of the diarrhoea.

In one of the other positive cases the serum reaction was positive for a period of one week and was not present again until death occurred two weeks later. Again, it was present from the seventh day until death on the twenty-second. In another instance it was complete in one hour, in dilutions of 1–500, for a period of two months, when the child died, the stools having been formed and good for three weeks. In one case which recovered without serum treatment the blood gave a

positive reaction for sixteen days, and in another infant for four weeks.

The bacilli were most numerous in the stools containing blood and mucus, but they were also found in those which consisted of fecal matter with only a small amount of mucus.

Rosenthal (1) found the Bacillus dysenteriae not present in the stools later than the twenty-first day, and that in but one case. In the present series the organism was isolated from the colon at the autopsy three weeks after the onset of the disease, in two cases. It was not present at autopsy nineteen days after the onset in one case, and in the third week in two others. During life it had disappeared on the twenty-fourth day from the stools of one child. In two cases treated with "Harris" anti-dysenteric serum, the bacilli were not present on the fifteenth day (nine days after the injection) and on the twenty-first day (fifteen days after injection) respectively. The stools were good. In a third case the bacilli were found in the stools twenty days after the serum had been given and twenty-nine days after the onset of the disease. They had disappeared two days later. That they may be present for a period of two months after the onset of an attack may be inferred from the following case: Among the children examined as a matter of routine in the gastro-enteritis ward at the Foundling Asylum, was a girl of ten months in fairly good condition. Her stools were yellow, smooth, and good, with a trace of mucus occasionally. From one such the Bacillus dysenteriae was isolated, rather to the surprise of everyone. Her blood gave an excellent reaction in 1–100 with the Flexner ("Harris") bacillus; not with the Shiga nor with B. coli communis. Two months before the present ob-

(1) loc. cit.

servation she had had an attack of diarrhoea for three weeks, with four green and mucus stools per day. No blood was passed at any time. The child went out to board, and six months later was reported to be in good condition, without intestinal disturbance during that time.

The Bacillus dysenteriae was not found at autopsy in the heart's blood nor in any viscus in five of my cases, which died. A septicaemia due to the Bacillus dysenteriae seems to be a rare occurrence. Rosenthal (1) found one such case among fourteen autopsies. Duval and Bassett succeeded in cultivating the bacillus from the mesenteric glands and liver in only one case. (2)

Infection in the hospital occurred in several instances, both at the Foundling Asylum and at the Babies' Hospital. One acute case is of especial interest: C. S., a female infant of ten months, convalescing from pneumonia, was well nourished, without fever, and doing well. There were three cases in the ward from which the Bacillus dysenteriae had been isolated, when the child developed diarrhoea quite suddenly, accompanied at once by marked prostration and a temperature of 103° F. The stools were small, never more than four in number, and consisted of green fecal matter, mucus, and a few specks of blood. From one of the stools, on the second day, the Bacillus dysenteriae was isolated with ease, the colonies being numerous on the plates. The blood gave a negative reaction on the second and third days, positive in 1–50 on the fourth and sixth. Death came on the seventh day.

The second case (J. M.), was that of a poorly developed child two years old. There was no history of

(1) loc. cit.

(2) loc. cit.

any diarrhoea, and she had one soft, brown stool a day. At this time the stools of every case in the ward were being examined in order to determine whether the Bacillus dysenteriae were present in cases which gave no symtoms. Only gas producing bacilli were isolated in her case however. Two days later her stools became thin, yellow, four in number, with mucus and a little blood. The Bacillus dysenteriae was isolated on the first day and the child's blood gave an excellent reaction in a dilution of 1–50. It continued positive, to the day of her death two months later, in dilutions of 1–500, blood and mucus having disappeared from the stools three weeks before. The blood reactions were obtained with various strains of the Bacillus dysenteriae from other cases in this series, but with the Shiga, colon, typhoid, and paratyphoid bacilli the child's blood was invariably negative in 1–50.

In two cases of pneumonia occurring on the floor below that on which the dysentery cases were kept, the stools became frequent (seven per day) and contained much mucus, but no blood. Dysentery bacilli were present in large numbers. Death occurred three and twelve days respectively after the onset of the diarrhoea. In the absence of any positive previous history it is impossible, of course, to state whether the dysentery occured as a terminal infection, as is rational, or whether a previous infection was stimulated to an exacerbation by the intestinal irritation coincident to the attack of pneumonia.

In one case in the Babies' Hospital, a child (B. A.) with pneumonia developed loose, mucus stools, as many as nine per day. The Bacillus dysenteriae was found, but the blood gave a negative reaction for fourteen days, when "Harris" serum was administered. The child recovered, the diarrhoea lasting eighteen days.

A child (J. C.) with bronchitis, from whose green and mucus stools the Bacillus dysenteriae was readily isolated, was discharged cured of her bronchitis, but with the dysentery bacillus still in the stools, ten days after admission. She was visited at her home and found to be in good condition, without and symptoms of intestinal disturbance. The significance of cases like this and B. C. as a source of infection in tenements is evident.

Four other cases occurred during convalesence from pneumonia. A marasmic child (C.B.) acquired dysentery in the hospital, recovered from it, and is now gaining in weight. His blood gave a positive reaction during a period of four weeks.

While examining every patient in a ward in which five positive cases had been found, a two year old girl came under observation. She was in good condition, and the stool examined was normal. The dysentery bacillus was not found in it. The history gave no record of any diarrhoeal illness, but the stool was said to have been thin occasionally and to have contained a little mucus. Her blood gave a rapid and perfect reaction in thirty minutes, and during the seven weeks that she remained under observation the blood continued to react in dilutions of 1–500 with every strain of the Manila type of the dysentery bacillus in my possession; but no agglutination was obtained with the Shiga, typhoid, colon, or paratyphoid bacilli. There had undoubtedly been a (clinically) mild infection some time before. Repeated examinations of the stools proved negative.

To sum up:—Cases of infection with the dysentery bacillus in infants tend toward the clinical picture of dysentery, with frequent mucus and bloody stools.

In some cases of hospital infection, occurring as terminal to other diseases (especially pneumonia) and in

other mild cases, the stools may never contain blood, but mucus is present in every case and usually in large amount.

The serum reaction is uncertain during the first week, frequently negative after the sixth day, but may be absent for two weeks. It cannot be relied upon for early diagnostic purposes in infants and young children.

The isolation of the Bacillus dysenteriae from the stools is the only positive evidence of infection during life.

The bacilli are present in the stools for a period of two to three weeks but may remain for a longer time.

The type of the organism almost invariably found in New York City is that of the "Harris Manila" or Flexner bacillus.

THE PATHOLOGICAL ANATOMY OF A SERIES OF CASES OF INFANTILE DIARRHOEA IN WHICH THE DYSENTERY BACILLI WERE PRESENT.

JOHN HOWLAND, M.D.

Dr. John Howland reported the results of autopsies on seventeen young children suffering from infection with the Bacillus dysenteriae. In the majority of cases bacteriological examinations were made ante-mortem; in three cases where the condition had not been suspected during life the bacteriological study was post-mortem. The organisms found corresponded in all cases to what is known as the Flexner Manila or indol producing dysentery organism. In eight cases cultures were made from all the organs at autopsy; but in no case was the bacillus

found except in the intestinal tract. In one case, the liver contained streptococci. The other organs remained sterile or showed only the colon bacillus, with the exception of the lungs which often showed a great variety of organisms. Fifteen of these seventeen children developed the infection while in institutions; two were suffering from the disease at the time of admission. The cases could be divided clinically into four classes: acute, sub-acute, cured, (death being due to another condition developing later) and terminal infections. There were no chronic cases. The acute cases were those which were fatal in ten days or less from the beginning of the characteristic intestinal symptoms. These cases were more apt to show blood in the stools than were the sub-acute cases and were accompanied by a more marked rise in temperature, though this was not the invariable rule. There were seven acute cases in this series,—one showing a pseudo-membranous enteritis; one a marked necrosis and ulcerative process; and one in which there was merely intense congestion with beginning ulceration and necrosis. The detailed study of the post-mortem findings showed that as a result of the infection with the dysentery bacillus there was a great variety in the pathological conditions found. Acute cases and terminal infections showed usually very marked congestion, more or less deep necrosis and ulceration. The cellular infiltration of the mucosa was irregular, but in general the more acute the process the more pronounced the infiltration. The same was true of the oedema and infiltration of the submucosa. The infiltration of the mucosa was largely polynuclear, while in the submucosa mononuclear cells predominated. In only two cases was the muscular layer affected and then but slightly. The peritoneal coat was involved in only one case. In one of these acutely fatal cases the

lesion was what would be usually considered as sub-acute; that is, slight congestion, no ulceration, except through the follicles which were excavated and showed moderate hyperplasia. Of the three cases classed as sub-acute by reason of the duration of the disease, two exhibited the same lesions as the acute cases, though to a less degree; while in the third the hyperplasia and depression of the follicles was the prominent condition and the mucous membrane was not necrosed. Microscopical examination of the tissues for organisms gave nothing characteristic. In the necrotic areas, bacilli negative to Gram of the proper form and size were found in enormous numbers, both in the acute and in the sub-acute cases; but cocci and bacilli positive to Gram were also very abundant. Usually the organisms were present in considerable variety and did not penetrate deeply into the mucosa; but in one case they were present in the superficial portion of the submucosa. Both ileum and colon were usually affected, changes in the latter being most marked. In three cases, however, the ileum suffered the most, and in one of these, a pseudo membranous case, there was hardly any change in the colon. It is interesting to compare at this time these cases with the Manila ones in adults described by Flexner, the specific germ being the same. He found the histological lesions in the mucosa to consist chiefly of coagulative necrosis with exudation of fibrin and polymorphonuclear cells. In Dr. Howland's cases the necrosis and the polynuclear cells were present but no fibrin could be demonstrated though special staining was done for this. Flexner found the submucosa always much altered, swelling, hemorrhages, fibrinous and cellular infiltration, and he said that the changes might take place beneath an intact or almost intact mucous membrane. In contrast to this, the cases described by Dr. Howland

showed no hemorrhages or fibrinous infiltration and the swelling and accumulation of cells never took place except beneath a very much altered mucous membrane. The conclusions in regard to the organisms agreed; though in one of the speaker's cases bacilli were found in the submucosa where Flexner has said he never found them.

From a consideration of these seventeen cases it appeared that in those terminating acutely, marked congestion, ulceration, or superficial or deep necrosis is the rule even though the disease had lasted as short a time as two days, while in the sub-acute cases the changes were more catarrhal in character with an increased follicular hyperplasia: in other words, the pathological findings were determined more by the severity of the infection than by the length of time that the disease had lasted,—a severe and overwhelming infection producing destructive changes rapidly, the clinical course being correspondingly acute and short; while a less severe infection was the cause of catarrhal processes with slight destruction, the clinical course being correspondingly long.

Discussion.

Dr. Philip Hanson Hiss congratulated Dr. Wollstein and Dr. Howland on the interesting reports which they had made. There were several questions which he would like to ask Dr. Wollstein. She had spoken of the organism falling into two groups: one group similar to the Shiga bacillus, and the other large group composed of organisms able to ferment many carbohydrates, —mono-, di- and poly-saccharids—with the production of acid but not of gas. He would like to know how Dr. Wollstein had made her fermentation tests and what the

source of her sugars had been. In the few cases which he had had the opportunity of examining he had found two groups of organisms, leaving the Shiga type out of the question, and he considered that unless the fermentation tests were very carefully made and the purest sugars were used that this difference might not be shown. Maltose at the best was very uncertain and all the forms of organisms if left long enough in it would probably give rize to fermentation. These two groups were distinguished from the Shiga organism by the fermentation of mannit and from each other by the fermentation, in the case of the more active fermenters, of dextrin and saccharose. The less active group fermented mannit but could not utilize dextrin and saccharose. Dr. Hiss thought the careful study and separation of these organisms very necessary because this report included a large series of cases histologically studied, and if anything was to be made out about the connection of these organisms with the different types of disease this was the only way to do it. Dr. Hiss considered it not unlikely that in other series of cases of dysentery and diarrhoea studied in this country, the organisms had not in all instances been preserved, as it had been thought, from the methods of study then used in their identification, that they were of the true Shiga and Kruse type. Hence, the frequence of occurrence of the different groups and their relation to the different types of gastro-intestinal disease in which they occurred could not now be determined. He therefore wished to ask Dr. Wollstein how she had made her fermentation tests and whether she had attempted to establish the etiological relationship of the different groups of organisms to the various types of disease which Dr. Howland had determined.

Dr. Augustus A. Wadsworth asked Dr. Howland

if he had used any stain beside the Gram for demonstrating the organisms in the lesions. Were any of the tannic acid stains used?

Dr. James Ewing said that while it was perhaps a little late to raise a question regarding the existence of infection by the bacillus of Shiga in many cases of infantile diarrhoea, yet it seemed to him that the relation between the disease and the bacillus had not been fully demonstrated in these valuable papers. It seemed to be a matter of importance to know in what relative abundance the Shiga bacillus had occurred in the stools of these infants, since it was probably not the only organism in the stools. It would have been of interest to know the results of a systematic study of the serum reactions of these patients, not only on the Shiga bacillus, but on the colon bacillus and other micro-organisms which might have been isolated. Perhaps the serum of some of the patients might have proved quite as active on other bacteria of the stools as on the variety of Shiga bacillus isolated and tested. It appeared that much work of this sort was needed to definitely fix the relation of intestinal bacteria to these intestinal diseases.

Dr. Wollstein said that the sugar reaction were made with the sugars obtained from Merck and had been made in two ways. The glucose, lactose, saccharose, mannit and maltose reactions were made in fermentation tubes as well as in litmus agar prepared without meat. The other sugars were used in the sugar-free litmus agar. In regard to Dr. Ewing's remarks, Dr. Wollstein said that she did nor think that it had been absolutely proven that the dysentery bacillus had been the only etiological factor in these cases and that she had therefore taken pains to call her report simply "The Dysentery Bacillus in a Series of Cases of In-

fantile Diarrhoea." The dysentery bacillus had certainly been present in the stools of thirty-nine cases, in greatest numbers in the cases with much mucus and blood. In the stools which contained more fecal matter they were often very few and difficult to isolate.

Dr. Ewing asked whether in any case the dysentery bacilli had been more numerous than other bacilli.

Dr. Wollstein said she had a case under observation at the time in which she thought that they were the most numerous forms present.

Dr. Hiss said in regard to the sugars, especially dextrin, that he had had specimens from Merck marked "highest purity", which in fermentation tests were little better than glucose and had acted practically as did glucose in all differential tests. Much dependence could not, therefore, be placed upon sugars bought in the open market, and it was necessary to test them thoroughly before depending upon results obtained with them.

Dr. Harlow Brooks asked Dr. Wollstein whether any other typical cases of dysentery in which the Shiga bacillus was not found had occurred in the wards during the time the cases reported had been under observation, and on what characteristics she based her diagnosis of dysentery in all these cases.

Dr. Wollstein said that if by typical dysentery was meant cases with much blood and mucus in the stools, there had been none.

Dr. Howland said that he had stained the bacilli with Gram-Weigert and methylene blue. He had not assumed that all these bacilli were dysentery bacilli and had therefore called them "bacilli of the typhoid-colon form negative to Gram". Nor had he assumed that in all these cases death had been due to dysentery.

CRETINISM IN A CAT.

F. H. MILLER, M.D.

Dr. Miller presented this case because of the extreme rarity with which it was observed in veterinary medical practice, this being the first case he had seen in fifteen years' of practice. The head of the animal was suppressed in growth and was very wide across the forehead in proportion to the size of the cat. There was a condition of exopthalmos which was very marked. The under jaw was about one-third short of the length of the upper jaw and the tremendous thickening of the bone made it impossible to close the mouth. There was no ankylosis. The animal showed the short limbs and over development of the muscular tissue very unlike that of rachitical patient. Dr. Miller said that he had seen the animal for the first time when it was about two months old. While, of course, the head had since grown a great deal, it had been even at birth very abnormally large, having the great width between the eyes. The cat had been examined by several doctors and oculists, and injections of atropine into the eye had shown a great difference between it and the normal eye. The periphery of the optic nerve was very pale and the nerve disc was very markedly depressed. It was important to state that the intelligence of the cat was very subnormal. Its life was entirely a matter of comfort and taking things easily. The pelvis was normal, and the great difficulty was in the limbs from the knee and elbow joints downwards. The metacarpal bones were more or less normal. It was impossible to find any trace of the thyroids though careful examination had been made. There was probably either obliteration or total absence of them.

Discussion.

Dr. Stuart Hart asked whether the thyroids were palpable in the normal cat.

Dr. Miller said that they were readily found, especially in a cat of this size. He believed that in this case they were entirely wanting. The cat could take food only with great difficulty. There was a tremendous thickening of the facial bones. The teeth were set in a semi-cartilaginous bone, not true bone at all.

CASE OF RHABDOMYOMA OF THE TONGUE.

HARLOW BROOKS, M.D., AND DR. STEBBINS.

Dr. Brooks and Dr. Stebbins reported a case of rhabdomyoma of the tongue, which was so far as they knew the only case ever reported, the tumor being very rare in striated muscle. The patient was a Hebrew, born in Russia but resident in this country for some time. The family history was negative. Two years before he had had a broken tooth which projected, and irriated his tongue on that side until it raised a small nodule which he had had cauterized and cut off with a pair of scissors. The tooth had then been taken out. After this time the enlargement had given him no trouble, though he could still feel it, until six weeks before the last removal when it had begun to enlarge. The nodule measured about .7 cm. across, .6 cm. in width, and .3 cm. in depth. It was solid and very firm. Microscopically it did not show the typical form of rhabdomyoma such as was generally found in the organs of the genital tract. Nearly all rhabdomyomas have originated from the organs arising from the intermediary cell mass, so that this case had a double interest. Some of the tumor cells showed striation fairly well. There was no doubt but that the cells were those of striated muscle though most of them were of a embryonic type. They had gone beyond the stage of unstriated muscle fibers. The case was particularly inte-

resting because the tumor arose from an all together different embryonal source than the ordinary rhabdomyoma.

THE PRODUCTION OF DIPHTHERIA ANTITOXIN.

WM. H. PARK, M.D.

Dr. Park gave a preliminary report upon some experiments showing the rapid formation of diphtheria antitoxin in horses following one or more injections of a large amount of diphtheria toxin which had been previously neutralized by antitoxin.

Two series of horses were injected. One group received toxin to which six times the amount of antitoxin necessary to neutralize its toxic action in the guinea-pig had been added, the other received toxin to which one and one-half times sufficient antitoxin to neutralize had been added.

Results in two horses receiving one and one-half times as much antitoxin added as sufficed to neutralize the toxin so that it did not injure the guinea-pig.

Horse No.	Amount of toxin injected in fatal doses for 1 gram of guinea-pig with toxin neutralized one and a half times.	Number of injections	Lapse of time	Amount of antitoxin developed per c.c.
221	100,000,000	1	8 days	1 unit
222	100,000,000	1	8 "	60 units
223	100,000,000	1	8 "	20 "
214	210,000,000	3	25 "	275 "
215	210,000,000	3	23 "	100 "
216	210,000,000	3	22 "	150 "
217	210,000,000	3	22 "	175 "
	With toxin neutralized 6 fold.			
211	210,000,000	3	25 days	6 units
213	210,000,000	3	23 "	1 unit

The results of injecting these large amounts of toxin partially neutralized by antitoxin have both a practical and a theoretical interest. In an emergency antitoxin of a good grade can be obtained from at least 50% of the horses within a period of three weeks. With more suitable neutralization it seems possible that this time can be shortened. This formation of 60 units of antitoxin in horse 222 in eight days from a single injection, and of 275 units in horse 214 in 25 days from three injections proves that it is not necessary, as Ehrlich has supposed, that cells should be adopted through successively increasing injections of toxin to produce free antitoxin in large amount, but that at the time of first encountering the toxin the cells can produce antitoxin as rapidly as at any future time. The difficulty has been that with toxin to which no antitoxin has been added, it is impossible to inject sufficient toxin to stimulate suitable cells to produce antitoxin in large amount without at the same time killing the animal by the effect of the toxin on these or other cells.

The lack of any great formation of antitoxin in the horses injected with the toxin to which excessive amounts of antitoxin had been added is striking.

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Dr. William H. Park, *President.*

THE PATHOLOGICAL ANATOMY OF THE SHIGA BACILLUS INFECTIONS OF THE INTESTINES IN INFANTS.

JOHN HOWLAND, M.D.

Dr. John Howland presented a paper to the Pathological Society on the pathological anatomy of the Shiga bacillus infections of the intestines in infants. The organism found in all the cases examined was the type known as the acid-producing or Flexner Manila organism; that is, it did not ferment glucose, saccharose or lactose,

but did split mannit with acid formation. It produced an early acidity in milk with a subsequent alkalinity, and agglutinated with the "Harris" serum in high delutions. Thirty-two cases were examined. From a study of these cases it seemed that they could be grouped with considerable accuracy according to the histological findings.

Group A, comprising five cases, represented the severe infections where the pathological changes were of a pseudo-membranous type. The membrane may be of irregular distribution or very extensive, involving both the colon and the ileum. The pseudo membrane was composed chiefly of necrotic tissue, desquamated cells and countless bacteria. Fibrin was present in very small amounts or not at all. There was a necrosis of the mucosa and it was this necrosis which chiefly gave the appearance of a false membrane; but in all these cases the necrosis did not extend beyond this coat. In the necrotic tissue and at its border there were hemorrhages, and thromboses, and it was divided from the healthy tissue by a zone of small cell infiltration, containing both mono- and polynuclear cells. The submucosa in four of the severe cases was much swollen and infiltrated.

The cellular infiltration was composed of cells resembling Unna's plasma cells and was most marked about the blood vessels. The muscular coat showed only a slight mononuclear cell infiltration. The peritoneal coat was normal.

Group B included four cases in which the mucous membrane in both the small and large intestines were in a good state of preservation. The changes occurred chiefly in the lymphoid follicles. The submucosa was normal as were the muscular and peritoneal coats.

Group C includes those cases characterized by

superficial necrosis and ulceration in the mucous membrane not limited to the follicles as in Group B, and not accompanied by the formation of a pseudo membrane as in Group A. In this group there were five examples. The changes consisted of congestion and hemorrhage into the mucosa with necrosis of isolated portions of the mucous membrane, which necrotic portions might be exfoliated so as to form ulcerations on the surface. The necrotic areas and ulcerations were usually surrounded by a zone of inflammatory reaction. The submucosa occasionally showed cellular infiltration. The other coats were not affected.

Group D contained fourteen cases which showed microscopically but few discoverable lesions, beyond congestion and moderate hyperplasia of the lymphoid tissue and, in one case, of slight cellular infiltration of the submucosa. There was very little histological change. As far as the other organs were concerned there were no characteristic findings. The mesenteric nodes were usually somewhat enlarged, rarely markedly so. The lungs showed hypostatic congestion and small areas of broncho-pneumonia. The liver was fatty in about the same percentage as in other infantile conditions. The spleen was as a rule unaffected unless some other infection was added. The kidneys showed cloudy swelling. Cultures from the organs did not show the Shiga bacillus. It is evident that the Shiga bacillus may produce a great variety of lesions and that with these lesions may be associated all grades and types of diarrhœal disease as distinguished by clinical symptoms and pathological findings.

RESULTS OF THE EXAMINATION OF SEVENTY-NINE CASES OF SUMMER DIARRHŒA.

CHARLES W. DUVAL, M.D., AND MR. E. H. SCHORER.

Dr. Charles W. Duval read a short abstract of the work which he and Mr. E. H. Schorer had done during the summer under the auspices of the Rockefeller Institute in the laboratory of the College of Physicians and Surgeons, on the subject of the bacterial flora in infantile diarrhœa with especial reference to the dysentery bacillus.

Seventy-nine cases of so-called "summer diarrhea" were examined, under which clinical title were included all types of the disease from mild diarrhœa to severe ileocolitis. The stools were examined within ten to fifteen minutes after they were passed and to this fact was due a large amount of the success obtained in isolating the Bacillus dysenteriæ. The bacillus was present in greater or less numbers in every variety of intestinal disturbance, and also in normal stools from infants. Of the cases of summer diarhœa, seventy-five, or ninety-four per cent, showed the dysentery bacillus.

The character of the stools was no certain indication of the presence of the Bacillus dysenteriæ, though the organisms were most likely to be isolated from cases showing bloody mucus. Plates from the stools showed a few scattered colonies or almost pure cultures. Five of the cases examined showed bacilli of the types "Harris" and "Shiga"; or, in other words, those which ferment or do not ferment mannit. In this same group of stools, the Bacillus "Y" of Hiss and Russell was also isolated. Five cases contained the two types. In three

the fermenting type predominated in the proportion of eight to three; in two the non-fermenting was present in greater numbers. In eleven cases the bacillus was of the non-fermenting type, or true "Shiga". In fifty-eight cases only the fermenting or "Harris" type could be found.

In the identification of the colonies after they had been fished from the plates, the semi-solid medium of Hiss was found to be superior to glucose agar, as it excluded motile organism and showed gas production in a few hours. In plating the stools it was found wise to select those parts which contained mucus and to shake them up in physiological salt solution or bouillon, then to set them aside for a few minutes to allow the coarser particles to subside, and to use one or two loops of the suspension for each plate, being careful not to disturb the settled particles. This method gave, as a rule, fifty to one hundred colonies per plate. Plates were incubated for from fourteen to eighteen hours and then every small pearl gray colony was subcultured to the Hiss medium. The colonies resembling Bacillus dysenteriæ were marked with a wax pencil and the plates were kept at a low temperature for a week, daily observations being made for fresh colonies. If the bacillus was not obtained in the first or second fishing all marks were erased and every small colon-like colony was transferred to the semi-solid medium. All tubes showing a cloud were disregarded. Those showing a growth only along the track of the needle were stirred with a platinum wire in order to bring out the bubbles of gas which had been formed. The cultures showing no gas production were tested for the agglutination reaction by suspending a few loops of the growth in the semi-solid medium in physiological salt solution and adding a known serum of high aggluti-

nating power. The identification of the bacillus by the agglutination reaction was not, however, wholly reliable as there are bacilli which react in high dilutions but which culturally do not correspond to the dysentery bacillus. The speaker laid great stress on the reaction in litmus milk observed over a period of weeks for the final identification of the organisms.

RESULTS OF THE EXAMINATION OF SIXTY-TWO CASES OF DIARRHŒA IN INFANTS.

MARTHA WOLLSTEIN, M.D.

Dr. Martha Wollstein read a paper in which she described the results of the study, during the three summer months, of sixty-two cases of diarrhœa in infants from two months to two years old, the stools of which had been examined for the Shiga bacillus in the Laboratory of The Babies' Hospital. Dr. Grace Dewey had assisted in the work. The material had been obtained from the wards of the The Babies' Hospital and of the Foundling Asylum.

Of the sixty-two cases, 47, or 78% were positive. At The Babies' Hospital the mortality rate was 55% of the positive cases and at the Foundling Hospital 78%.

From stools containing much mucus, with or without blood, the Shiga bacillus was isolated quite readily in most cases. Stools with little mucus and no blood often necessitated fishing of very large numbers of colonies on successive days before any Bacillus dysenteria could be found. In every instance it was the acid or mannit-fermenting variety of Bacillus dysenteria which was encountered in this series.

RESULTS OF THE EXAMINATION OF FIFTY-ONE CASES OF GASTRO-INTESTINAL DISTURBANCE IN INFANTS AND CHILDREN.

LOUISE CORDES, M.D.

Dr. Louise Cordes reported the results of an examination of the stools of fifty-one cases of gastro-intestinal disturbance in infants and children conducted in the laboratory of the New York Infirmary for Women and Children, in order to determine the presence or absence of the Bacillus dysenteriæ.

The material was obtained from unselected cases; even those showing the mildest symptoms were examined. It was, in a good many cases, obtained fresh and plated at once.

The Bacillus dysenteriæ was found in the stools of twenty-six cases, two of which were five and ten years old respectively. In twenty-five of the stools the acid mannit type of the Bacillus dysenteriæ was found. In one the alkalin mannit type.

Agglutination as high as 1 to 3000 and 1 to 3500 was obtained with the bacillus of the acid type from six cases. The same organisms showed an agglutination with the Shiga anti-dysenteric serum in from 1 to 200 to 1 to 500.

The bacillus of the alkalin mannit type gave a positive reaction with the Shiga anti-dysenteric serum in a dilution of 1 to 7500. With the Harris anti-dysenteric serum a fair agglutination was obtained at 1 to 2000.

The blood of 45 of the cases was tested with the Harris and Shiga dysentery bacillus in dilutions of 1 to 40 or 1 to 50. In ten a positive reaction was obtained.

THE RESULTS OF AN INVESTIGATION UPON THE ETIOLOGY OF DYSENTERY AND ACUTE DIARRHŒA.

WILLIAM H. PARK, M.D., KATHARINE R. COLLINS, M.D., AND MARY E. GOODWIN, M.D.

Dr. William H. Park reported the results of investigations carried out by himself, Dr. Katharine R. Collins and Dr. Mary E. Goodwin upon the etiology of dysentery and summer diarrhœa at the Research Laboratory, the work having been carried on so much in common that a single joint report was made, as follows:

In general our results have agreed with those just reported by Drs. Wollstein and Cordes and differ somewhat from those of Dr. Duval. We have not found the Shiga type of bacilli at all in the ordinary summer diarrhœas. We have paid especial attention to the agglutination of the two groups of dysentery bacilli. The agglutination test for the identification of the dysentery bacilli is one to be carried out only with the greatest care. The test properly performed is delicate and reliable, but when carried out with insufficient precautions is misleading. It has been known for some time that the blood of animals before immunization, possessed in moderate amount substances which agglutinated many bacteria. An agglutination of bacteria must therefore take place in high dilution of serum before it can be considered a specific reaction. From results we have obtained in the Research Laboratory we consider that even this requirement is insufficient for testing dysentery bacilli. We found before any inoculations had been made, that the blood serum of certain horses agglutinated, even in such high dilutions as 1–1000, both the bacilli having the characteristics of the true dysentery bacillus

of Shiga and those having the characteristics of the mannit fermenting class of Kruse, Flexner and Duval. This applied to a less extent to the serum of grown goats and even in a very slight degree to the serum of rabbits. It has happened in same instances that after repeated injections with cultures of one of the varieties of the dysentery bacilli, that the animal's blood agglutinated the variety of bacilli not used in immunization in as high or even higher dilutions than it did the variety injected.

We found that a serum drawn from a horse which had been immunized by repeated injections of the dysentery bacillus received from Shiga, agglutinated this bacillus in dilutions of 1–500. The same serum, however, agglutinated the mannit fermenting bacillus in dilutions of 1–1000. The serum from another horse which has received injections of the mannit fermenting bacillus, agglutinated this bacillus in dilutions of 1–1000; but also agglutinated the Shiga bacillus in dilutions of 1–500. These two different varieties of bacilli would be judged by these reactions to be much alike in their affinities. Indeed, Shiga and Flexner seem to have made this mistake. Duval and Bassett certainly fell into this error, when they announced in the Fall of 1902, that the mannit fermenting type from the Baltimore diarrhœa cases and the Shiga type had identical agglutination characteristics. These varieties are now known to differ completely in this respect. Young animals before immunization were found by us to be wholly or comparatively free from bacterial agglutinins and in them we were able to produce an almost pure development of specific agglutinins. A goat six weeks old was found by us to possess before treatment no appreciable bacteria-agglutinating substances in its blood. After four injections of a

culture of Shiga dysentery bacilli, its blood in dilutions of 1–500 agglutinated Shiga bacilli, but only in dilutions of 1–10, the mannit fermenting variety. A rabbit, whose blood was negative before immunization, after six injections of this bacillus agglutinated the mannit fermenting bacillus in dilutions up to 1–5000. The Shiga bacillus was agglutinated only in dilutions of 1–20. The slight development of substances which agglutinated the mannit fermenting type during the process of immunizing to the Shiga bacillus cannot be considered as showing any affinity between the Shiga type and the fermenting type, as about the same increase was found in animals injected with nutrient bouillon and other substances. It seems to us that the general employment of immunized horse's serum for differentiating varieties of dysentery and colon bacilli is a mistake.

The true dysentery bacilli of Shiga were found by us in ten cases of characteristic dysentery occurring in different parts of Mount Vernon and Tuckahoe. These towns are situated just north of New York City. The bacilli were also found in a serious case occurring in the Coney Island district of Brooklyn. The mannit fermenting bacilli were found in about one-half the cases in the Mount Vernon epidemic, in five cases among workmen on Riker's Island, in two cases at Orange, in one case at Coney Island, and in three cases in New York City. All above cases exhibited characteristic symptoms of dysentery and differed in no way from cases where the true Shiga bacilli were obtained. In no characteristic case of acute dysentery where we obtained suitable material for examination did we fail to find either the Shiga or the mannit fermenting type of dysentery bacilli. Besides the characteristic cases of dysentery in which as a rule some history of infection was obtained, a large number of

cases of summer diarrhœa were met with in which excessive mucus with or without traces of blood occurred in the stools. In the majority of these cases the mannit fermenting type of dysentery bacilli were found, although at times in very small numbers. In none of these were the Shiga bacilli discovered. In cases of cholera infantum, no dysentery bacilli of either type were met with. They were also not obtained from the stools of cases of subacute diarrhœa in institutions where no cases of the dysentery type existed. The blood from cholera infantum and simple diarrhœa cases did not clump any of the dysentery bacilli in our collection in dilutions higher than 1–10. The Shiga type of bacilli obtained by us from three different localities were found to agree closely in cultural characteristics, in their agglutination, and in their sensitiveness to a specific bactericidal serum with the cultures of the same type obtained from Shiga (Japan), Duval (New Haven), and Wollstein (New York City). The mannit fermenting bacilli from the characteristic cases of dysentery and from the cases of summer diarrhœa agree in most respects with each other and with the cultures obtained by Flexner in Manilla and by Duval in Baltimore. They differ quantitatively and possibly also qualitatively in their reactions to the specific agglutinins and bactericidal substances produced in animals after injection of different cultures. From the Shiga bacilli they differed wholly in their reaction to specific agglutinins and bactericidal substances. The writers believe that it would simplify matters to designate the bacilli identified by Shiga as dysentery bacilli, and the mannit-fermenting-indol-producing bacilli, as para-dysentery bacilli. The Shiga variety is found rarely except in the characteristic cases of dysentery or in milder cases associated with them,

while the mannit indol type is found in a moderate number of characteristic cases, but most often in the irregular mucus diarrhœa of summer. The relationship between these two groups of bacilli is very similar to that between the typhoid bacillus and the para-typhoid bacillus.

Discussion.

Dr. Park asked Dr. Duval if he did not think that a serum, such as the immunized horse serum which frequently agglutinated both types of dysentery bacilli in high dilutions, as well as other bacteria, was dangerous to use for purposes of identification.

Dr. Duval replied that Dr. Park evidently misunderstood his paper, that he identified the organisms culturally and then tested every culture with the sera "Shiga" and "Harris." All his isolations were agglutinated by dysentery serum.

The serum he used was that prepared at Philadelphia by Dr. Gay. Horse serum used, agglutinated in dilutions of 1–1000 or more. The main point was to determine first the cultural features, then try the agglutination reaction. Even if a positive reaction is obtained with the horse serum in 1–50, 1–200 or 1–500, the bacilli cannot be identified as dysentery unless followed out over a period of several days, with special reference to litmus milk, to identify them by their cultural features.

Dr. Libman said that the work reported indicated that the bacilli were found mainly in the cases with mucus or mucus and blood, and that it seemed that every loose stool with mucus was accompanied by the organisms in question. Such a condition of affairs, that is, a constant

etiological factor for a pathological secretion, was unknown elsewhere in the body. So that the question arose whether the mucus was not a favorable medium for the growth of organisms which might be present normally, in at least some of the cases. He was aware that the organisms had not been found in normal stools; but a crucial test had not been made. The contents of different parts of the bowel should have been examined in children that had died of non-diarrhœal diseases. While he did not wish to doubt the relationship of the organisms in question to diarrhœal diseases, a doubt would remain until such studies as he suggested were made. It was possible that organisms which existed normally in the upper part of the bowel might be found in the stools in diseased conditions. Tissier had shown that a dose of calomel was enough to produce a stool full of streptococci in cases where none or very few had been present before. He said he had, several years ago, found in two cases of cholecystitis, bacilli corresponding culturally to the dysentery bacilli. Dr. Libman believed that one could not say that a bacillus which when isolated did not agglutinate with a specific serum was not a dysentery bacillus. One ought to make several tests, for it might be possible that such an organism might react after it had been cultivated outside of the body. He and others had had such experience with the typhoid bacillus. Dr. Libman asked Dr. Duval what his experience had been with the thread reaction, whether he had found it more or less specific than clump reactions.

Dr. Duval replied that his bacillus did not differ from the true Shiga type except fermenting in mannit. The kind of reaction (end-to-end or tight clump) depended on the media and serum employed. Sometimes a fresh Shiga culture would give an end to end reaction

and sometimes the Harris culture would give it. No hard and fast rule could be laid down.

Dr. Park said that in most of the diarhœas with profuse serous discharge bacteria were very abundant. In a dysenteric stool from a man working on Riker's Island, which was almost pure blood, very few bacteria developed.

Dr. S. J. Meltzer wanted to know whether watery stools had ever been examined for their bactericidal effect. Such stools, being transudates from the blood might possess acquired bactericidal power, specific for the organism which is the primary cause of the dysentery. Hence the absence of just this group of bacteria in the watery stools.

Dr. P. H. Hiss said that he had noticed a tendency in the papers read that evening and during the discussion to refer to the dysentery bacilli as of "acid" and "alkali" types, and to speak of "acid" and "alkalin" dysenteries. He thought that it was not scientifically correct to divide these organisms into "acid" and "alkali" types, as both of these types produced acid freely if in the presence of a suitable sugar. So far as he knew, in the presence of proteid and nitrogenous food alone, one of these types would give rise to as much, or as little, alkali as the other. It did not, therefore, seem to him correct to speak of an "alkali" producing type, simply because that type did not ferment as many kinds of carbohydrates as the so-called "acid" type. Dr. Park had suggested the name "para-dysentery" for the group composing the active fermenters or "acid" type, in order to distinguish it from the true Bacillus dysenteriæ of Shiga. These active fermenters had already been shown to be divisible into two fairly distinct types not only by their fermentation reactions but by differences in agglutination. In

view of these facts and our as yet really limited knowledge of these organisms and their relation to dysenteries and diarrhœas, it seemed early to attempt to name them or at least to mass them indiscriminately under one group name. At present we knew only something of their action on carbohydrates, practically nothing of their action on proteid or other nitrogenous food materials, and the further study which was neccessary might lead us to a different conception of the members of the group.

Dr. Duval also objected to the use of the terms "acid" and "alkalin" groups. He preferred to distinguish them by the terms "fermenters" and "non-fermenters".

Dr. Park asked whether the same objection might not apply to the use of the term "para-typhoid".

Dr. Hiss thought so. He was against naming groups of bacilli until more was known of their characteristics.

Dr. Park said that prefixing the term "para" simply separated that group from the Shiga variety and did not necessarily limit the mannit fermenting bacilli to a definite group but left that to the future to decide. Most of the bacilli so far obtained were, however, in most characteristics, similar. Some of the German bacteriologists had used the term "pseudo-dysentery" for this group, but this was misleading. He agreed fully with Dr. Hiss, but said that the terms "acid" and "alkali" were simply terms of convenience and referred to the appearance of the differentiating litmus mannit medium.

Dr. Herman Schwartz asked Dr. Duval whether he found bacilli in watery stools where no blood or mucus was present, or in true summer diarrhœa.

Dr. Duval said he had.

Dr. Harlow Brooks said he was confused as to just what was meant by dysentery now-a-days.

Dr. Park replied that the term "dysentery" was a distinctly clinical term. It referred to an acute infectious disease lasting from one to four weeks, having the distinguishing symptoms of abdominal pain, tenesmus, and frequent stools of blood and mucus with more or less feces and serous fluid. These cases were rarely seen in New York City but occurred by hundreds in country towns. It was imposible to examine the mucous membrane of the intestines in these cases during life. From the location of the pain and the character of the stools it was, however, possible to determine pretty accurately the extent of colon and small intestine involved and the character of the inflammation.

Dr. Brooks thought these cases corresponded clinically to cases which would not have been described as dysentery before the discovery of the bacillus.

Dr. L. Emmett Holt did not think we were much helped in the classification of intestinal diseases by the researches reported. For instance, such cases of colitis as were found by autopsy to have large quantities of pseudo-membrane had always been included in the group, dysentery, but were frequently without blood and mucus as clinical symptoms. To exclude them from the classification on this basis seemed irrational.

Dr. Park said that the uncomplicated cases of dysentery outside of hospitals usually recovered in this climate, and when fatal an autopsy could rarely be obtained. The autopsy findings in some atypical hospital cases due to the mannit fermenting type in which extensive membrane had been present, had been carefully examined by Dr. Howland whose report had just been read. It was very difficult to determine in these cases what portion of the lesions should be ascribed to

the poison of the dysentery bacillus. In these cases no true Shiga dysentery bacilli had been found.

Dr. O. H. Schultze said that croupous colitis did cause death. Some of those cases died where there was no blood and no mucus in the stools.

Dr. Park said that it was a recognized fact that different micro-organisms produced inflammations resembling each other in many respects. Thus, we had syphilitic inflammations of the pharyngeal mucus membrane and also lesions due to Vincent's bacillus which closely resembled in appearance the lesions of diphtheria. We also had the slighter lesions due to the streptococci. Notwithstanding these facts no one doubted the value of recognizing the most important and frequent infection of this region as diphtheria. The same reasons existed for the term "acute dysentery."

Dr. George P. Biggs said that the term "dysentery" referred simply to a group of clinical symptoms and had no place in pathology. The pathological lesion in dysentery is a colitis which apparently varies in type and degree.

Dr. David Bovaird said that there were three distinct points of interest in the discussion.

1.—We could not tell from pathological lesions which cases were Shiga bacillus infections or were associated with other bacilli.

2.—We could not tell from clinical picture which cases were produced by Shiga bacillus and which were not.

3.—Nor was the agglutination test alone reliable evidence as to which cases are produced by the Shiga bacilli.

Practical workers had no other means than the bacteriological for recognizing these cases. We must

wait for further information from bacteriologists before making definite distinctions either as to the bacteriological association of these cases, or as the prospect of treatment with specific serum.

Dr. Park said he could judge in what cases dysentery bacilli would be found as well as he could where typhoid bacilli or diphtheria bacilli would be met with. They would always be found readily in cases of epidemic dysentery and usually wherever acute diarrhœa was accompanied by mucus and blood. In cholera infantum they would not be found. In less characteristic cases of the type of summer diarrhœa they were sometimes met with, and sometime not, just as diphtheria bacilli were sometimes found and sometimes not found in slight throat exudates.

Dr. Schwartz thought it was going to far to say that summer diarrhœa was due to the dysentery bacillus. When blood or mucus preponderated the bacilli were found without difficulty; but in commoner cases of summer diarrhœa, even if a few colonies were found, one could not say that the dysentery bacillus was the cause of the watery stools.

Dr. Duval wanted to know if with the bacilli in one of the eight colonies from such a case there was reaction with that patient's blood 1–50, 1–500, 1–1000, and all other organisms in the intestinal tract were not agglutinated, what importance would Dr. Schwartz attach to that finding.

Dr. Schwartz replied that he would consider such a finding of great importance.

Dr. Goodwin wanted to know what success Dr. Duval had had in finding bacilli where the diarrhœa extended over a month, two months or three months.

Dr. Duval replied that it was very difficult to isolate the bacilli and that one might work with several specimens from one case and then find very few.

Dr. Schultze thought we were departing from the old rule in ascribing the finding of these bacilli to a clinical picture and not to the pathological lesion.

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Dr. William H. Park, *President.*

Dr. Cyrus W. Field read a paper on Typhoid Bacillus Infection of Oysters.

This work was undertaken at the suggestion of Dr. Park to determine whether or not the oyster could be the means of typhoid infection and if so, under what conditions it would act in this manner.

After various experiments it was found that the best method of infecting tanks in which the oysters were placed for observation was to sow the Bacillus Typhosus on agar slanted in flasks, this was incubated for 24 hours, at the end of this period the growth was washed off with normal salt solution; this was poured into the tank and more fresh salt solution added to the flasks to wash off the remaining bacteria. The oysters were removed at the

ends of certain periods and were examined bacteriologically. At each period five oysters were taken from the tanks, washed and scraped to remove as many organisms as possible from the shell. 1–10 c.c. of juice was used to infect each plate; at the end of 48 hours 20 to 40 colonies were fished from each plate. They were planted in bouillon and at the end of 24 hours were examined in hanging drop and a strongly agglutinating typhoid serum was added.

The dilution used was 1–800. If the organisms in the tube did not agglutinate they were thrown out and no further tests were made upon them. If they did they were planted in lactose agar and milk.

In the latter part of experiments he relied entirely upon the agglutination test. No organisms were found agglutinated in this series that did not react as Bacillus Typhosus to the other tests.

No. Oysters	No. Bacteria tested per c.c. in infect. tank	No. hours in infected tank	No. bacteria in infected tank	No. bacteria in oysters per c.c.	% of B. Typhosus in oyster
5	20,000	24	0	1,200	22%
5	14,000	48	0	900	18%
5	8,000	96	0	750	9%
5	6,500	7 days	0	570	7%
5	4,200	9 "	0	490	1%
5	4,600	14 "	0	300	0

In the next experiment, oysters were taken from infected tank at the end of 24 hours and placed in an uninfected tank—fresh salt water same as that in the Aquarium, used with all fishes.

5	19,500	24	0	1,300	19%
5		24	0	650	5.5%
5		48		300	3%
5		96		240	
5		7 days		200	
5		9 "		210	
5		14 "		190	
		21 "		200	
		28 "		190	

Never later than the end of the 2nd day could the organisms be found in fresh water. This was due to the great dilution in the fresh water tank and their disappearance.

On taking the sea water used in these experiments and infecting it with B. Typhosus it was found that the organisms disappeared entirely from the water from the 6th to 8th day. 50% were destroyed at the end of 24 hours.

Some oysters were removed from the infected tank and kept on ice; no diminution was found in the number of bacteria present per cubic centimeter up to the 8th day.

The question is, does the oyster ever come in contact with infected material, and if so, does not salt water dilute and destroy the organism? That is, if taken from deep sea beds and brought to fresh water. Oysters are removed from beds and taken to small or large creeks and deposited at low tide in drinking or fattening houses, built in the stream at its edge and as the tide rises the house is filled with water which covers the oysters. As the water rises, one can hear the hissing sound made by the oyster in sucking it up. The specific gravity of this water is less than they are accustomed to and by a process of osmosis the oysters are enlarged, making them fat.

The streams may be grossly contaminated by sewerage. Should a case of typhoid occur in town near the creek and these oysters become infected by discharges from the patient, those eating the oysters would have typhoid fever, as was the case in Middletown in 1894. This epidemic was traced to oysters fattened in a river. Patients lived on the stream near the drinking house and discharges were drained into the river. Wherever the

oysters were shipped a typhoid epidemic followed. This condition of drinking houses is still the same in many places where oysters are shipped to the market.

The only way is to forbid the sale of fattened oysters, or the establishment of oyster beds where the the water might be contaminated, or the process of fattening where the water was or could be contaminated.

Discussion.

Dr. Wm. H. Park said that if by the work of Dr. Field, and further experiments, it could be definitely proven that the bloated or fattened oyster was a source of typhoid infection the Health Department would take the ground that oysters in New York sold in the shell, should not be oysters bloated or fattened in creeks, which are more or less grossly contaminated with town sewerage which flows into the creeks.

It is fairly easy for anyone used to it, to detect the bloated oysters from those taken from the deep sea beds. Those who care most for oysters, do not care for the bloated or fresh water variety; they prefer those from the deep sea, which seems to be safe.

Dr. Manges asked Dr. Field if the fattened oysters had any distinguishing marks, and what was the percentage of their sale in New York.

Dr. Field replied that there were no distinguishing marks to be seen except by opening the oyster. If they were fat and plump and filled the shell, which normal oysters did not, they were almost sure to be fattened. The more reputable dealers would not handle them. Oyster connoisseurs would not touch them. They would detect the lack of the brackish taste of deep sea oysters.

He had visited one of these creeks and asked a native if the water was used for drinking purposes?

"No", the latter replied, "sewerage goes into that, we would not dare drink it." Dr. Field said, "Do you eat the oysters?" He said, "No, those go to the New York market." They are also kept there for the convenience of oyster shippers who don't want to go to the beds with their boats to get them. They bring in great quantities of them, and store them in houses as they are called. Then as wanted, put them in barrels and ship by freight to New York. They are sometimes 24 to 48 hours in transit.

Dr. Manges said that at Mt. Sinai Hospital there were a large number of typhoid cases each year, but for Mosaic reasons oysters were not consumed by many of these patients. Outside of the hospital in cases of typhoid fever he always made a point of asking about oysters. Wherever typhoid had been found in cases of oyster eating they had been eaten outside of New York. He did not know of any cases arising in this city. He did know of cases of oyster infection coming from places in the neighborhood—Long Branch and Atlantic City. Two years ago at the latter place the water where the oysters were fattened was a splendid culture medium from sewerage contaminations. As far as New York oysters went, he thought we were pretty safe. Everything suspicious in oyster culture should be prohibited and if the Board of Health would make known the point emphasized by Dr. Field, how to tell the fattened oyster, it would afford oyster lovers satisfaction and protection. Many were afraid of the oyster on accont of this agitation.

Dr. Park said that Dr. Field had told him that on account of newspaper talk about the typhoid infection of oysters, that the demand had fallen off one-third.

It was true we did not know of any such cases in New York, but we could, by such safeguards, make the

use of oysters perfectly safe. Not interfere with their use, but rather allow people to use them.

Prof. G. N. Calkins gave a demonstration of the results of his studies on the bodies found in the lesions of variola. The bodies seen were the same as those originally described by Guarnieri and Pfeiffer, and more recently by Councilman, who had placed the material for the present study in Prof. Calkins' hands. It had been fixed in Zenker, preserved in alcohol, and stained by the method described by Borrel in the *Pasteur Annals*, *1901*, *p. 49*. Three possible cycles of development of the organism have been traced, the most important of which, the sexual cycle, we owe to the observations of Prof. Councilman. This is intra-nuclear. The first cycle is a simple growth with segmentation into gemmules, which then infect fresh tissue cells. In the third, which also appears to be a sexual cycle, pansporoblasts are formed, which break up leaving a Restkörper. Vesicular bodies, similar in appearance to those described in the epithelial cells in variola, are also seen in carcinomata and sarcomata, and in the tissues infected by diphtheria, but the varioloid bodies can be distinguished by the fact that a complete series can be traced.

Discussion on Dr. Calkins' remarks.

Dr. Tyzzer gave a brief outline of his work. He said he had made a comparative study of the lesions following vaccine inoculations and the specific inclusions which accompany those lesions. In taking up this work before being fully convinced that these inclusions were

organisms, he followed out various hypotheses. In superficial examination, these inclusions may resemble various sorts of degenerations. He sought to trace these inclusions back to included leucocytes, red blood corpuscles, or degenerations of the protoplasm of the cell. After studying a great many lesions he had been unable in any case to find any form which links the typical characteristic inclusion with any of these degenerations.

In vaccine lesions he had found that they formed the same series as given by Dr. Calkins for the cytoplasmic forms. The organisms at first lie between cells, become larger by growth, take their position near the nucleus, and finally undergo segmentation into new forms.

Concerning nuclear forms, he found none, although degenerating and disintegrating nuclei were found. The experiments had been made upon the tissues of rabbits and various other animals on cornea, skin and mucous membrane. It was quite evident that these inclusions bore some resemblance to degenerations, but after a study of lesions in these various animals and in the various tissues of these animals, he still considered that they are organisms.

In the course of this work lesions were studied from a series of one hundred rabbits, several guinea pigs, two sheep, twelve calves and three adult cows.

Dr. W. R. Brinkerhoff spoke briefly of work done by him with Dr. G. B. Magrath, under the direction of Dr. W. T. Councilman, upon the lesions of experimental variola in the monkey. They found many of the forms described by Prof. Calkins in the lesions of small-pox in man, the work being strongly confirmatory of the work of Prof. Calkins.

Mention was also made of a degeneration of certain

cells of the testicular epithelium in acute diseases which led to forms simulating a certain stage of cytoryctes. He then pointed out the grounds on which such a degeneration product could be differentiated from the parasite.

Dr. Anna W. Williams said that before Dr. Councilman's very important paper appeared, their section work was mainly with rabbits' corneas inoculated with vaccine virus.

Since his paper, they had begun the study of serial sections from monkeys inoculated with variolous virus from different cases of variola. This work was practically just begun as they had studied sections from only two monkeys, and she therefore felt some hesitation in speaking about it. She wished to mention, however, one or two perplexing points.

Their two monkeys showed a great difference in reaction to the inoculation of the virus. In one, the eruption developed late and was of a mild form. In this one, very few definite intra-nuclear changes were found. In the other monkey, an early and abundant eruption developed, and both the intra-cellular and intra-nuclear changes were marked. In sections from this monkey they had found nearly all of the forms described by Dr. Calkins, but they had not yet been able to trace out the definite, consecutive cycle described by him.

In the first place, there seemed to be frequently beginning vesiculation with no bodies present either in the vesiculated cells or in those in their immediate neighborhood. Then the intra-cellular forms showed an extreme irregularity in the time of their appearance and in the size of the amœboid forms and in the size and shape of the segmenting forms, more of an irregularity than Dr. Calkins had described in his remarks.

Of course they knew that they might have degenerative changes in such a protozoon as well as in the host cell, and that such degenerative changes might occur more frequently in one monkey than another, giving at times a very complicated picture. Such a condition might account for some of the peculiar intranuclear changes which they had found. They had been able to trace a series of apparently undoubted degenerations into which some of the forms shown by Dr. Calkins seemed to fit.

A substance appeared in the nuclei in advancing lesions which stained a homogeneous, brilliant pink, with hæmatoxylin and eosin, and a greenish blue with magenta and indigo carmine.

This substance first appeared in small, irregular masses, often by the size of the principal chromatin mass of nucleus. As the lesion advanced, this substance was often seen partially and then completely surrounding the chromatin mass, leaving that as a small, more or less rounded central body which takes the chromatin stains as a rule brilliantly. Such a picture seemed to resemble one of the first stages of the primary sporoblasts as described by Dr. Calkins.

Other nuclei were more or less filled with the homogeneously staining material, most, however, still retaining a chromatin mass. Many of these later nuclei contained in this homogeneously staining material, irregular granules, some highly refractive, and others not, some larger with a vacuole-like center approaching definite ring bodies in appearance and some of these with a central granule. It was thought by the speaker that these appearances might be due to mixed protozoon infection and degenerative changes, but so far, they had

been unable definitely to say that they were due to more than degenerative changes.

Dr. James Ewing said that he had heard Dr. Calkins' presentation three times and felt that it had become more convincing in each repetition. From the very first he had been greatly impressed by the examination of the specimens and had felt that we were probably face to face with the solution of the problem of variola. He had never been quite free from doubt, however, regarding the interpretation to be placed on the intra-nuclear forms, while in regard to the extra-nuclear vaccine bodies he believed that no one could afford to ignore the generally accepted opinion of pathologists that these bodies represent degenerative products. The line of proof thus far brought forward to demonstrate the protozoon nature of all these bodies, intra- and extra-nuclear, seemed to him incomplete. Very little or no attention had apparently been paid to the possibility of reproducing the intra-nuclear forms by other methods. In his own study of vaccine bodies he had become familiar with and overwhelmed by the host of structures in necrosing variolous lesions which recall the forms of protozoa, and early concluded that it would be impossible to demonstrate the presence of a definite protozoon cycle on the basis of morphology alone.

During the past year he had been attempting to ascertain if vaccine bodies varied in the different animals known to be susceptible and in any others that might be made to react in any way to inoculations of vaccine, in other words to establish a comparative morphology of vaccine bodies. He had in this way compared vaccine bodies produced in man, monkey, rabbit, frog, and mud-puppy.

The same type of extra-nuclear body, in general,

seemed to be produced by vaccine in each of these animals. One could hardly believe that the same protozoon was capable of growth in animals so distantly related as man and batrachian. He thought that the appearance of the vaccine body in so many different animals could with difficulty be reconciled with the protozoon theory, and seemed to indicate rather that this body represents a universal function of the diseased epithelial cell rather than a particular micro-organism invading the cell.

Early in 1903, Sikowsky, a Russian observer, reported that vaccine bodies in the cornea could be reproduced by inoculation with diphtheria toxin. In repeating this work he had been greatly surprised to find that within 24 to 48 hours after inoculation of the rabbit's or frog's cornea with diphtheria toxin practically the same changes were produced as by vaccine. The very numerous forms of bodies produced resembled in striking fashion those seen in variola and vaccine. Some of them were totally different in type from anything he had ever before seen as a result of degenerative processes and it would have been difficult to have imagined them to be of such origin had any other conclusion been possible. In tracing the origin of these bodies he had come to the conclusion that they originated from a peculiar form of coagulation-necrosis with the production of a semi-fluid elastic material which by comminution and vacuolation reproduced many of the protozoon-like forms of vaccine bodies. This necrotic process involved either the nucleus, or the cell body, or both. It produced minute or large rings and globules, intra-nuclear or extra-nuclear, single rings or congeries of vacuoles, rosette-like bodies, and very peculiar cyst-like bodies large and small. Many of them he found indistinguishable from similar bodies he had seen in variolous lesions, and strongly resembled the bodies included in the cycle of the variola parasite.

In drawing conclusions from these observations he felt one could not safely claim that no protozoon forms are mixed up with the vaccine bodies in variola, but they did seem to show that the morphological evidence so far presented had failed to demonstrate any protozoa in variola, and that morphological studies alone if limited to variola would probably always remain indecisive.

Prof. Calkins said that he felt quite sure that the bodies he had just described were parasites and not degenerations.

Dr. Ira von Gieson thought it seemed very curious that the problem, which has occupied so much attention for the last twenty years, should be opened up by so simple a process as the ordinary technic of cutting and staining sections made from sores. He thought so comprehensive a problem would have been worked out on these lines long ago. He thought the great living problem of the day was the action of the cytolytic agents, which he thought, as time went on, would be found to constitute the major part of the process. The organism itself, he said, has about the same relationship to the process as the spark has to the train of gunpowder which it ignites. The finding of the organism does not explain the rest of the process in so extremely complicated a sequence of phenomena as that which we call variola. He thought the organisms probably merely carriers.

Dr. Herman Schwartz read a paper* on the results of the bacteriological examination of the stools from cases of summer diarrhœa with the special object of finding the

* Read at the meeting of October 14, 1903.

Bacillus dysenteriæ. He took only those cases of typical summer complaint which were characterized by profuse watery stools with little or no mucus. He first examined the blood of twenty-five healthy infants for agglutination reactions with Bacillus dysenteriæ in dilutions of 1 to 10, 1 to 20, and 1 to 50. These, with one exception, 1 to 10, were all negative. The normal stools of three healthy breast fed and three healthy bottle fed babies were then examined with negative results.

The stools from thirty cases of the character as stated above were also studied.

Technic.—The stools were taken by means of double tubes; the anal region being disinfected, the larger calibre tube was inserted about one-half inch in the rectum; the second tube of smaller calibre and longer than the first, was inserted through this larger tube, well into the rectum and the mucous membrane of the latter irritated. This seemed to be the only feasible method of obtaining these watery stools. When macroscopical mucus was present the fluid stool was put into a Petri dish and the mucus fished out and put into bouillon. Otherwise, a few c.c. of this fluid was put into bouillon and then in thirteen cases agar plates in the ordinary way made. In the remaining seventeen, the method used by Dr. Duval was used. In all the thirty cases the results were negative. The blood of these cases was also examined for agglutination reactions (1 to 10, 1 to 20, and 1 to 50) with negative results.

Conclusions.

1. The blood of healthy infants and children rarely contains agglutinins for the dysentery bacillus.

2. The normal stools of infants in the cases examined did not show the presence of these bacilli.

3. That in the cases of typical summer diarrhœa with green, watery stools, these bacilli were not present, or if present, must be in exceedingly small numbers.

4. The serum from these cases of summer diarrhœa showed no agglutinins for the dysentery bacillus.

5. From the results of these observations, it would seem that these bacilli are not usually a factor in this class of summer diarrhœa.

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Dr. William H. Park, *President.*

NOTES ON A NUMBER OF MALLEIN REACTIONS.

Dr. Letchworth Smith said it was the purpose of these notes to present as briefly as possible some of the results of examinations of a number of horses killed during the summer after having been tested by mallein.

A reaction to mallein included three points:—

1. General reaction: Dejected appearance, staring coat, etc.

2. Local reaction: Appearance of tender swelling lasting several days.

3. Rise of temperature. According to the Health

Department Circular of Information regarding the use of mallein, this was "from 2.7° to 7.2° F.," while French and German authorities insist it should be at least 3.6° F. or 2° C.

The list of cases given did not include all the horses killed, but those which fullfilled the following conditions:—

1. Tested by mallein during the months of March, April, May and June, 1903, and notes of the tests recorded.

2. Condemned as result of tests.

3. Autopsied and bacteriologically examined by Dr. Schwarz, Dr. Weil and Dr. Smith.

The results were interesting on account of the temperatures the part of the mallein reaction which usually claimed the greater share of attention. The temperature was taken five times in the twenty-four hours preceding injection of mallein and five times following it. The mallein was made by Ravenel's method.

In estimating the rise of temperature during these tests, the average taken before the injection was compared with the average taken after it, rather than by comparison of the highest temperatures reached in each case.

Of the 43 cases with conditions as above mentioned, eight had shown a rise of 2° C. or more in temparature. Of these, three showed lesions interpreted as glanders, besides yielding the glanders bacillus; two showed indications of glanders but the bacillus could not be isolated. Three seemed normal.

In 22 cases of the 43, there was a rise of 1 to 2° C. Six of these were bacteriologically and pathologically proven to be glanders. Seven were pathologically positive. In nine cases there were no indications of glanders.

In the thirteen remaining cases the temperature rose less than 1° C. Four yielded glanders bacillus, five showed suspicious lesions and four were negative. Even in the cases in which there was a marked rise of temperature, more than one-third showed very doubtful lesions, or none whatever.

As the foreign authorities would lead one to expect, thirteen cases with temperature changes less than 2° C. were negative.

But in the attempt to "stamp out" glanders, the destruction of normal horses was no more important than the mistake of passing over one having the disease. If the conservative course had been followed—of considering the cases negative if they failed to show a rise of temperature of 2° C.—ten horses from which the bacillus was recovered and twelve others which were very probably cases of glanders, would have remained alive, and been a possible source of infection.

The speaker regretted that more reliance could not be placed on the negative reaction. Some cases put down as negative might have proven positive bacteriologically if the destruction of the horses could have been satisfactorily managed. It was impossible to get possession of material as soon as desired.

Dr. Smith spoke of the lack of knowledge of lesions which simulate glanders. The experience of the summer supported the position of those who consider mallein a favorable diagnostic agent in suspected cases of glanders rather than those who condemn it.

Whether or not under ordinary circumstances the actual destruction of horses, which showed no symptoms of glanders except a reaction to mallein is justifiable, was still an open question.

The later observation of Nocard, Babes, and other

authorities on the curability of the disease tended toward the more conservative idea of isolation until the appearance of more definite evidences that the animal is useless.

Dr. Harlow Brooks reported an Unusual Case of Multiple Neuritis. The patient was a woman, born in Russia, 50 years of age. Apparently there was no tuberculous, specific or neurotic taint in the family. At an early age she began to drink whiskey steadily, but never to the point of intoxication. She also drank a little beer, tea and coffee, but not to excess.

She married when 20 years of age and from this time forward had various symptoms which reappeared from time to time. Her ankles became painful and swollen after exertion, pain disappearing after rest. She had attacks of diarrhœa following periods of constipation, the latter lasting six or seven days. At 40 years of age she was frightened and after this had headache which lasted for a year and a half, but was relieved by domestic remedies.

At the age of 41 her ankles became so swollen and painful that walking was impossible and she remained in bed for a time after which she was able to walk about. Because of painful callosities on the sole of her feet she walked on the heels which gave her a peculiar gait which still persists at the age of 50. Two and a half years ago she noticed some anesthesia of the soles of the feet. This was not permanent but remained for a week or two and then disappeared only to return again.

Two years before her admission she had lumbago with severe and persistent pains in the wrist. She lost

control of the bladder for nine months when she recovered completely. During her last illness of nine months' duration, she suffered from pain in the larger joints of the body, was very weak, could do no housework and remained in bed much of the time. She had no control over urination and defecation. Pains in the back were very severe, also pains in feet, arms and shoulders, and she could not raise either her hands or feet. After six months in bed with intervals of improvement, she was able to sit up without help.

When examined at the Hospital the muscular dexterity was impaired, myotactic irritability was wavelike, there was no perceptible enlargement of the superficial lymph nodes. She showed no mental weakness, was good natured and complained very little of the pain from which she apparently suffered. The muscles were flabby and normal in volume, except for a slight wasting of the small muscles of the right thumb. Sensibility as to pain, touch, and temperature normal.

When two fingers were placed upon the arm, she recognized four, usually increasing rather than decreasing the number of points. Reflexes were exaggerated, motor power diminished. Slight foot drop was noticed when patient was in bed. The patient developed a bed sore, grew rapidly worse and died.

Post-mortem showed the lesions of a long-standing case of alcoholism of the chronic type. The brain was abnormally firm. The spinal cord was firm in spots, in other places it was soft. No apparent changes in the retina.

Anatomical diagnosis was peripheral neuritis probably of alcoholic origin. There was interstitial hyperplasia in all the organs. In the brain, spinal cord and nerve roots, it was found that the cortical fibres coming

from the large pyramidal cells of the frontal and parietal regions of the cortex were extensively degenerated. The fibres were also degenerated in the internal capsule, the medulla and spinal cord showed a marked descending degeneration most marked in the crossed pyramidal tracts. As was expected, degeneration of the posterior tracts was found but it was more extensive than anticipated. There were, however, no distinct areas of sclerosis.

The posterior root ganglia showed cytoclastic changes and both posterior and anterior nerve roots were extensively degenerated. In the peripheral nerves there were interstitial and parenchymatous degenerations more prominent in the smaller trunks less so in the larger. Presence of the descending degenerations was most interesting. These degenerations were present without marked mental change as though the motor fibres were particularly affected and yet in the so-called psychic areas sections showed some degenerated fibres.

The speaker thought it rare in cases of alcoholism producing degeneration of the brain, cord and peripheral nerves, to find the descending degeneration fully as marked as the ascending changes. He thought the lack of knowledge of these brain changes was due to the fact that observers had been satisfied on finding the changes in the peripheral nerves and did not search further for alterations in the brain.

Discussion.

Dr. M. G. Schlapp said that two years ago he had shown a case of so-called alcoholic neuritis in combination with mental symptoms (Korsakow's syndrome) to the Pathological Society, in which there had been marked changes in the spinal cord and medulla. The brain of

this case had not been examined up to the time of the presentation, but later he examined and found changes throughout the cortex. This case differed from the one of Dr. Brooks, in that the mental symptoms were very marked.

The point brought out by Dr. Brooks that these changes had not been observed in similar cases of alcoholic poisoning before, is very likely due to the fact that in examining these cases former observers did not use a method whereby acute degenerative changes, such as are found in alcoholic poisoning, are made visible. These changes are demonstrated by the Marchi method.

The lesion as a rule is the result of an acute or subacute condition. It does not involve whole tracts, but only groups of fibres in certain tracts; particularly the fibres in the posterior root zones of the spinal cord, and the intramedullary fibres of the sensory cranial nerves.

The speaker agreed with Dr. Brooks, and wishes to lay special stress upon the statement, that it is very likely that in all those cases of so-called alcoholic neuritis, the lesion is not confined to the peripheral nerves but is also found in the central nervous system, in fact in all the cases examined by the speaker, five cases of alcoholic neuritis with mental symptoms (Korsakow's syndrome) the lesion was more marked in the cord or brain than in the peripheral nerves.

Dr. J. E. Welch demonstrated a Patent Foramen Ovale and a Case of Multiple Serositis

In the first case the patient was an Italian boy of 16, who could not give an account of himself in English. He had dyspnœa and œdema of the legs which graduall

spread over the body and became general. The principal symptom was great pain over the precardial region; so great that he requested another Italian in the ward to give him some instrument with which he might commit suicide.

Autopsy showed heart greatly enlarged on the right side, hypertrophied and dilated. Deposit of recent fibrin over right auricle and ventricle and about apex. All valves were thickened, tricuspid and mitral to a much greater extent than the others. The patent foramen ovale was of enormous size, 1 by 1½ inches in diameter; just beneath it was another and smaller opening.

In regard to the second case:—In 1846 Van Deen reported a case with the following findings: Obliterating pericarditis, pleurisy with adhesions obliterating both pleural cavities. Inflammation of the peritoneal cavity causing general thickening of the peritoneum with marked thickening over liver, spleen and the intestines. This case had constant ascites with edema of the legs subsequent to the ascites.

Van Deen's theory was that obliterating pericarditis was the primary lesion and that the inflammation spread to the pleural surface, thence to the abdominal cavity.

Since then many cases have been reported. Dr. Wood of Philadelphia in the *American Journal of Medical Science, January, 1903*, showed that a constant feature was an ascites, the serous membranes throughout thickened with obliterating pericarditis. The principal point is the fact that the ascites appears before the edema in the legs in a great majority of cases. There is no change in the liver except in a few cases that would account for the ascites.

In Dr. Welch's case the patient was a stableman, aged 35 years. Was alcoholic. Took five or six glasses

of whiskey a day, one or two before breakfast, and enormous quantities of beer. Had had gonorrhea several times. Twelve years ago had chancroids followed by suppurating inguinal adenitis. Was never ill before. November 1st, 1902, cramp-like pains all over the body which soon localized in the upper part of the abdomen which became swollen and tender.

Physical examination showed that he had ascites with swelling of the legs and a rapid, feeble heart action. There was some increase of tension in the arteries which were small in volume. Somewhat cyanosed; no jaundice. Directly after admission to the Hospital a general syphilitic rash broke out. He then admitted having had a hard chancre which had healed five weeks previously. He was dismissed from the Hospital December 12, 1902, feeling much improved, and was treated in the Dispensary until the first of March, 1903, when he began to have swelling of the abdomen with pain; about two weeks later had dyspnœa and œdema of the legs. He entered Bellevue again, the 15th of March two or three weeks after his trouble began. Shortly afterward he was tapped, and a large quantity of fluid was drawn, which was of the nature of a transudate. He was tapped at intervals until just before his death, which was in the early part of August, 1903.

Autopsy showed (membranes of brain not examined, as permission to do so could not be obtained), obliterating peridarditis. The right pleural cavity contained firm, old adhesions about the base and 300 c.c. of serum, the left contained firm adhesions to the posterior border of the lung and about the base, three litres of serum. Had perihepatitis, (sugar-coated liver), perisplenitis, (sugar-coated spleen) and general diffuse thickening of the peritoneum. There were a few slender firm cord-like ad-

hesions between diaphragm and upper surface of liver over both lobes. There was complete obliteration of the great omentum, and gastrocolic omentum. and free communication between the greater and lesser peritoneal cavities. There were a few firm cord-like adhesions between stomach and transverse colon, tubercles in the lungs and in the bronchial lymph glands, few in the spleen, none in peritoneum or liver. Liver cirrhosed, inter- and intralobular connective tissue. There were 4,000 c.c. of turbid serum in the peritoneal cavity.

Cause of condition, probably alcoholism, syphilis and tuberculosis; tuberculosis in the peritoneum was not noted in this case. That is also given as a cause by some writers on the subject.

Discussion.

Dr. E. Libman asked Dr. Welch if there had been any positive venous pulsation; the combination of a mitral insufficiency with an open foramen ovale usually produced it. He said he had looked up the records of the hospital; in the last hundred autopsies there had been thirteen cases in which the foramen ovale had been more or less open. They had occurred at all ages from three months to forty-four years, and had not shown any clinical symptoms, that could be referred to the foramen ovale. Ordinarily this condition did not seem to produce clinical symptoms, unless combined with other congenital or acquired lesions, or particularly in infants with diseases of the lung causing interference with the circulation. In the case reported by Dr. Welch the opening was very much larger than he had ever seen.

Dr. Welch replied that there was a venous pulsation in the vessels of the neck and there was also a very distinctly pulsating liver.

Dr. Brooks said the case had been very carefully examined in the ward, and the lesion had not been classed as of the foramen ovale. The man arrived from the Steamboat and diagnosis could not be made out. The trouble was thought to be a double mitral lesion and possibly tricuspid. There were so many murmurs they could not be localized as coming from any one place.

Dr. George A. Tuttle reported a Group of Seven Cases of Paratyphoid Infection.

On the 14th of October four cases from the same family were brought to the Presbyterian Hospital suffering from what was thought to be typhoid fever. They were taken ill at about the same time one on the 5th of October, one on the 6th, one on the 7th, one on 8th or 9th.

The patients consisted of mother and three children, a daughter and two sons. The daughter was taken ill first. She was 17 years of age. She had not been well all summer, and had lost considerable flesh. Had more or less cough, and there was a suspicion that she might have tuberculosis. Occasionally had night sweats, no hemoptysis, palpitation of the heart, dyspnea on exertion, edema of eyelids, severe headaches, vertigo, vomiting, cramping pains in abdomen, some shaking chills during the summer, when she had nausea and vomiting, with profuse sweating afterwards.

These attacks lasted only a short time. Was feeling as well as usual until the 5th of October, nine days before she came to the Hospital, when suddenly she developed a severe headache, then nausea, vomiting, severe shaking chill, followed by fever and sweating, and

two days later, another shaking chill with fever and sweating. Since then had had irregular diarrhœa.

Was in bed for nine days previous to her entrance to the Hospital with these symptoms, with increased prostration, some diarrhœa, no epistaxis, but some headache and slight cough.

Physical examination at time of entrance to Hospital: Well nourished, strong, slightly anemic, no apathy, no prostration. Tongue inclined to dryness; along the edges was red but coated with whitish fur down the center. Pharynx and tonsils congested. Superficial glands somewhat enlarged. Heart action was poor, rapid, and there was a rough low pitched presystolic murmur at the apex well localized. Pulse small, rapid and soft. Lungs practically normal a few rales at base and in the axillae. Liver normal; edge could not be felt. Spleen reached to the costal edge and on deep expiration could be felt about one-half inch below. Abdomen soft, not distended, but tympanitic, no tenderness, no mass could be felt.

The surface of the body showed a few fairly typical erythematous spots on the abdomen and chest. When she came in had a temperature of 104; for the next few days, temperature ran an average of 103°. On the 18th day the temperature came down to normal, nine days after her entrance to the Hospital. The normal temperature persisted for a week. Following a dose of calomel it rose to 104 but subsided in 24 hours and then the patient went on with a very slight afternoon temperature for ten days.

Widal's test was tried on the 15th, 17th, 19th, 23rd, 25th, 27th, 29th, 31st of October, 2nd and 7th of November without any reaction. Leucocytes 7,200.

The next case to become ill was a boy, aged 10. He

came home from school sick with headache, pain in shoulders, and went to bed, vomited three times. No epistaxis, no cough, bowels loose. Frontal headache lasted two days. When he came to the Hospital said he felt better than he had the week before. Had been ill eight days when he arrived.

The boy was fairly well nourished, good color, only moderate prostration and apathy, no dyspnœa. Tongue moist, edges red, heavily coated down the center. Superficial glands all enlarged. Pulse regular, rapid, good force, no tension. Heart normal. Lungs had a few rales over the apex. Liver reached to the free margin of the ribs; could not be felt. Spleen could not be made out to be enlarged. Abdomen soft, not distended, tympanitic, no tenderness. A few reddish erythematous very faint spots on trunk. On the 16th, two days after admission to the Hospital one dejection showed a small blood clot. Temperature when admitted 103; normal on the 14th day.

Widal reaction made without any result.

The day after this boy was taken ill, a brother 9 years of age was taken in the same way with severe headache, frontal, becoming general and that was practically all. No cough, vomited once or twice, bowels loose. He was poorly nourished, prostrated and apathetic. Tongue dry and brown. Teeth covered with deposit, lips cracked open and bleeding. Gums soft and covered with white patches. Heart normal, pulse regular, soft and somewhat dicrotic. Lungs normal, liver could not be felt, spleen percussed rather large; edge could not be felt distinctly. Abdomen flat, tympanitic, no tenderness. Over the thorax and abdomen, many faint pink spots. Spleen became large and palpable two days later. Temperature 102 at time of admission to the Hospital.

Dropped to normal on the 18th day. No Widal reaction obtained in this case.

The mother was the last to be taken ill, and furnished the clue to the disease. She was 39 years of age, worked in the Health Department Laboratory, handling specimens, slides, serum, etc. She fell ill on the 8th of October, the day following the day the last boy was taken. She began to have headache, and the bowels were at first constipated, later loose. On the 9th of October she had a shaking chill, cough and scanty expectoration, fever and sweating. Prostration and fever increased. The night before she entered the Hospital was wildly delirious, and tried to jump out of the window. Had another chill, became cyanotic, and her condition was very bad.

Was corpulent, prostrated, apathetic, stupid, perspiring profusely, extremities cold, with marked cyanosis on her entrance to the Hospital. Respiration rapid, and considerable subsultus. Tongue dry, heavily coated, cracked. Tonsils dry, congested, superficial glands not enlarged. Heart had a faint systolic murmur over the pulmonary area, otherwise normal. Base sounds of poor quality. Pulse irregular, rapid, poor quality. Vessel wall palpable. Some emphysema of the lungs and a few rales heard behind. Liver normal size, spleen did not appear to be enlarged. Edge could not be felt. Abdomen full, tympanitic, not distended. Skin practically normal, no spots. Had chill night after admission, was in condition of coma vigil, temperature rose steadily with slight remission, then rapidly until it reached 107.8 when she died. Urine contained 10.10% albumen; leucocytes 15,000 when she came in, 19,400 the next day. One Widal reaction taken on the 15th, negative.

Autopsy made ten hours after death. Lungs normal, rather dry. Heart normal. Liver normal with the ex-

ception of the gall bladder. Wall of the gall bladder thickened and containing a number of fairly large calculi, one of them engaged in the duct, forming a sort of ball valve; and instead of containing bile, it was filled with a viscid, light-colored fluid. Evidently some cholecystitis. Spleen soft, pulpy, weighed 6¾ ounces. Kidneys weighed 6 and 6¼ ounces, capsule free, considerable congestion. All the superficial veins were congested, the cortex was pale. There was nothing to indicate any chronic disease. The intestines showed the patches everywhere normal except in the lower 2 inches just above the ileocecal valve. Here there were four or five small ulcers the largest one-half inch in diamater. They looked as if they had been punched out of the mucous membrane, some reaching the peritoneal layer. The lower part of the small intestine and the entire large intestine were filled with blood.

Cultures were made from the spleen in plates and some bouillon tubes inoculated. The colonies looked like typical typhoid colonies, in Hiss medium, were a little irregular with spirals reaching out. Pure cultures were obtained. They were put through the different tests; in milk they produced no coagulation. In peptone solution no indol was produced; in lactose bouillon, no gas. It was thought that the case was one of straight typhoid fever, but as a final test a specimen was obtained from a case of typhoid in the Hospital, whose blood had a very powerful effect on typhoid cultures, but there was no effect on these bacilli, they were motile and looked very much like typhoid bacilli, but there was absolutely no reaction produced in a fairly strong dilution after three quarters of an hour.

Blood from the children of this woman, who had been ill, was sent for and tested and an immediate, power-

ful reaction was obtained. There was loss of motility and clumping as soon as they could be put under the microscope.

Later six specimens were obtained from the Board of Health, of paratyphoid organisms from different sources—Buxton, Strong, and others. With blood from the children, reactions were obtained with four of these.

Living on the same floor with this family was another family of four persons. At least three of these people were taken ill at the same time and in the same manner. These two families lived on the top floor of a tenement house and obtained water from a tank on the roof, into which water was pumped from the cellar. They obtained milk from different sources, and it looked as if probably the water was at fault; however, it had been examined and nothing discovered in it.

A specimen from one of the cases in the other family was obtained, through Dr. Park, and this gave a prompt Widal reaction with these bacilli in a dilution of 1–60. The bacilli have been tested over and over again with specimens of blood from typhoid patients which gave a prompt reaction, with typhoid bacilli, and there is absolutely no reaction. The group of cases is considered paratyphoid.

Discussion.

Dr. C. W. Field said he wished to say a word about the tank on the roof mentioned in Dr. Tuttle's report. It was built last year, and was made extra large, the owner thinking he might make a deal with the owner of the next house, and have the four top floor tenants supplied with water from the same tank. This could not be done, and this large tank was pumped full, the water allowed to remain without freshening until all used. In

this way when water was low, it became offensive, nearly black, filthy and dirty, as both families assert. The husband in the other family refused to drink it; he drank beer instead, and remained well.

In this family there were five members, of whom three became ill. A man caller came frequently during the month of August and drank considerable water during the evening. On the 7th of October he developed a disease which was diagnosed as typhoid in Yonkers. He recovered. He had temperature when taken ill of 104½. All symptoms corresponded closely with the group reported, and without doubt he had been infected from the same source as the others. All recovered within about four weeks from the beginning of the attack.

Dr. Park said the only people attacked were those who used the tank water. Out of a possible thirteen, eight became infected. There were seven or eight other families in the house, with no cases of the disease whatever.

Dr. Libman wanted to know what sort of serum was used which refused to agglutinate the bacilli from these cases, but did agglutinate typhoid? He wanted to know also what was the reaction of the bacilli in glucose media.

Dr. Tuttle replied that when it was found that the bacilli did not produce Widal with the ordinary typhoid, they were tested with the glucose medium. In 24 hours there was a very faint bubble of gas. In 48 hours there was abundant production of gas; no gas in lactose.

Dr. Brooks asked if the ulcers looked like typhoid, also what was the condition of the retro-peritoneal lymph nodes.

Dr. Tuttle said that no observations had been

made of the peritoneal lymph nodes. In his experience Peyer's patches had been irregularly distributed in this neighborhood. There were punched out ulcers in the mucous membrane, with no evidence of Peyer's patch around them. If the patch had been there it was all gone. The ulcers were in just about the area where one would expect to find the patches—within two inches of the ileocecal valve.

Dr. Brooks asked if they looked like typhoid ulcers.

Dr. Tuttle replied that they looked like some typhoid ulcers. They were in a late stage. So many typhoid ulcers occupy only part of the Peyer's patch; in these no evidence of the Peyer's patch could be seen. He also said in regard to the microscopic appearance of the bacilli in the hanging drop they did not look like typhoid bacilli, and did not act like them. They appeared to be shorter, often double and frequently were in long strings, almost like streptococci. After staining they looked like typhoid, but were markedly different in the hanging drop.

Dr. Field said that of those who had been ill in this group all but three, and those all children, had been in a run-down condition all summer,

Dr. Park said the cases were interesting, as the woman who had died had been engaged in cleaning up a great many typhoid cultures at the Research Laboratory, and it had been suspected that she had become infected in the laboratory. If the disease had not been proven to be tank infection and paratyphoid, it would have been hard to make it clear that she had not been careless in working at the Department of Health. This showed how misleading apparent evidence might be in many cases.

Mr. J. P. Atkinson read a preliminary note on precipitines formed by injecting animals with globulin derived from normal horse serum containing diphtheria antitoxin. The experiments were devised to see whether in animals injected with the globulin of normal horse serum and with the globulin of diphtheric antitoxic horse serum were developed anti-bodies which would act as a specific precipitine for normal horse serum globulin and for the antitoxic horse serum globulin respectively, thus affording a method for separating the active substance in diphtheria antitoxic serum from the inactive substance.

Globulin solutions were prepared from normal and diphtheria antitoxic sera by precipitation with magnesium sulphate and were purified by solution and re-precipitation until free from serum albumin and finally were freed from magnesium sulphate by dialysis. Chloroform was used to prevent putrefaction.

Two rabbits were selected for immunization, one being injected with the solution of normal globulin, the other with the solution of antitoxic globulin. After a number of injections had been given each rabbit they were bled and the sera from the clots were tested against diphtheria antitoxic serum with the following results:

Two samples of diphtheria antitoxin which had just recently come into the laboratory to be tested were diluted so that one cubic centimeter of the dilution contained 1–10 unit of antitoxin. Two portions of five cubic centimeters each were taken from each dilution and to them was added serum from each rabbit so that the globulin in each antitoxic horse serum was acted on by what we may call an anti-normal globulin serum and an antitoxin anti-serum which should react for both normal and

antitoxic globulin. After precipitation had taken place the precipitates were filtered off.

An amount corresponding to one cubic centimeter of the original serum dilution from each filtrate was mixed with ten fatal doses of diphtheria toxin and injected subcutaneously into guinea-pigs. All the guinea-pigs died within forty-eight hours.

The antitoxin dilutions were tested against diphtheria toxin to make sure that without the presence of the rabbit sera these antitoxins would protect against ten fatal doses of diphtheria toxin.

The toxin was also tested and found to be of the estimated strength. The action of both rabbit sera was again tested. In this experiment twice as much antitoxin as was necessary to protect against ten fatal doses of toxin was used instead of the exact neutralization dilution. The result was the same. Both guinea-pigs injected died within forty-eight hours.

It will be seen by these tests that a specific anti-body differentiating between normal globulin and antitoxic globulin was not found, for the rabbit injected with normal globulin solution produced precipitating substances for the antitoxic globulin.

One would infer from the results of these tests that normal globulin and diphtheria antitoxic globulin are affected by a common precipitine, while differing in the molecular arrangement through which neutralization of diphtheria toxin occurs. It also adds to the belief that diphtheria antitoxin is of a proteid nature.

Experiments were made to determine whether the globulin precipitating serum would throw down the antitoxin from the toxin-antitoxin and leave the toxin free. It did not do this and thus showed that a strong combina-

tion existed between the toxin and the antitoxin. Whether the toxin antitoxin combination as well as the free antitoxin was precipitated or whether it was left in solution is being now determined.

Dr. F. C. Wood said that he did not think it was justifiable in our present state of knowledge concerning the precipitins to expect that the reaction would be in any way sharply limited to the antitoxin. From his own experiments and those of others it had been shown that the blood of an animal immunized with a pure proteid, for instance, a serum albumin from human chest serum, would give a serum which precipitated not only human serum albumin but globulin and even haemoglobin. It was still doubtful whether the haemoglobin, for example, was actually precipitated by the antitoxin or whether it was carried down mechanically with the precipitum from other proteids present. It seemed to him also that the assumption from precipitin tests alone that diphtheria antitoxin was of proteid nature lacked foundation, especially after the results obtained by Jacoby in isolating the toxic group from ricin. Apparently the toxin in this case, though it could be precipitated by means of neutral salts, yet was not of proteid nature but only attached to the proteid molecule. The contradictory results obtained by Obermayer and Pick and Michaelis showed that there was much to be learned about the exact chemical nature of the precipitated substance.

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Dr. William H. Park, *President.*

SOME OBSERVATIONS ON LYMPHATIC LEUKÆMIA.

L. B. GOLDHORN, M.D.

During the past few years I have had the opportunity of examining the blood of a number of cases of Lymphatic Leukæmia; the last one of these presented such remarkable departures from all other ones that I reexamined such slides that I had and others lent to me for that purpose by some of my friends and I have now the honor of communicating to you the inferences I have

drawn, hoping that at least some of the facts will be of interest to you.

Before I enter upon the subject proper I wish to say a word about the morphology of the blood in leukæmia, or rather give my interpretation of such.

Leaving the pseudo-leukæmia infantum of von Jacksch out we have two distinct forms of leukæmia, which I will simply call lymphatic leukæmia and myelogenous leukæmia, omitting the usual addition of "spleno" or "lieno" on account of the not infrequent involvement of the spleen in the lymphatic variety.

From a hæmatological point of view, which I regard as the only proper one, these two are readily held apart; from a pathological or anatomical point that seems to be impossible.

If we divide the leucocytes into two great classes, the hyaline and the granular ones, we have in myelogenous leukæmia an increase in the granular varieties and in the lymphatic one an increase in the hyaline forms with the probable exception of the mononuclears and the transitionals. The myelogenous form is so to speak a new growth of those centres of the bone marrow which give origin to the granular leucocytes of the blood and the lymphatic form a hyperplasia of those centres which supply the blood stream with lymphocytes. Both can form metastases like malignant new growths, the pathological changes in the viscera depending upon them. Just as no two cases of carcinoma with metastases are alike so no two cases of leukæamia are alike.

Recognizing that leukæmia is not a disease of the blood proper it is to be expected that changes in the red blood and the plasma vary as the nature and the seat of the metastases vary.

This is the manner in which I have been able to ex-

plain to myself the enormous differences in the degree and character of the different cases of leukæmia.

So far I have never experienced any difficulty in telling the two leukæamias apart. In myelogenous leukæmia I look upon the mast cells as the sole characteristic cell; their enormous increase forms so constant a feature that they appear as pathognomonic, for while there are many diseases in which the myelocytes are increased, myelogenous leukæmia seems to be the only one in which these cells are so abundant as to give a characteristic aspect to the blood picture. This is not usually taught and the reason for it seems to have been inadequate technique in the demonstration of these cells. Before the invention of the Jenner stain the demonstration of the granules of these cells was a difficult or at least tedious matter. I would suggest for their rapid demonstration a saturated solution of a basic dye like Methylene blue or Dahlia in ordinary wood-alcohol. Their granules are brilliantly stained by applying this solution to the unfixed bloodsmear for a few seconds and washing in water.

I have been unable so far to obtain a bloodsmear from a case of undoubted myelogenous leukæmia in which I could not readily in this manner show this cell in enormous numbers.

In what has been termed a mixed leukæmia an enormous increase in the various granule bearing leucocytes was seen together with what appear to be large mononuclear hyaline cells; to me, having Ehrlich's classification of the leucocytes in mind this would not seem a leukæmia of double, viz.: myelogenous and lymphatic origin but one of purely myelogenous origin in which the mononuclear cells are not lymphocytes but the monuclear and transitional cells of Ehrlich. A mixed leukæmia would

under those circumstances appear to be of very rare occurrence, as rare as a carcinoma with a sarcomatous element in it.

The last case of lymphatic lenkæmia in which I had the pleasure of making the blood examination occurred in a lady 70 years of age. The case is one of Dr. Arthur Lyman Fisk's, who has kindly furnished the following notes on the physical examination. The spleen now reaches down to the umbilicus; in the latter part of October it reached only half as far. The leucocyte count then showed a hundred thousand less cells per cbmm. than it does now, but this is probably insignificant as the leucocytes fluctuate enormously in some cases even during the 24 hours. There is no glandular enlargement otherwise, though the liver reaches below the free border of the ribs. Patient suffered from diarrhœa and when this was under control patient improved steadily while splenic tumor increased.

I was surprised to find no distinct evidence of anæmia. There were no blasts of any kind, no poikilocytes, no washed-out corpuscles, none of the common degenerations, no increase in bloodplatelets, but a leucocytosis of 500,000. 97 to 98 per cent. of these leucocytes were lymphocytes. Eosinophiles, neutrophiles and mast cells were just about normal numerically, *i. e.*, per cbmm.

The great majority of the lymphocytes were small lymphocytes, showing but little degeneration. The hæmoglobin was high giving a high color-index, but it is well known that its estimation is not a simple matter in leukæmia. This is about the only condition in which I would recommend the use of the Talquist Filter paper book. The reason why I believe this book unreliable is that it gives in all cases of lessened oxygenation of the blood, and markedly in cases of cyanosis, a reading which

lies eventually much beyond the scale of the color comparison chart.

After carefully comparing the blood findings in this case with other cases I have expressed a belief that the prognosis in this case is good. To my great satisfaction Dr. Fisk tells me now of another similar case of leukæmia in which the splenic tumor reached to the symphysis pubis and which there was otherwise no demonstrable infiltration of lymph nodes. This case ran a very chronic course. A marked contrast to this case is one which I saw four years ago and which came to autopsy. The patient had a marked lymphæmia and an anæmia of exceptionally high degree. I had the pleasure of showing a preparation from this case to Prof. James Ewing because I found a number of undoubted megaloblasts besides some myelocytes. There was at the same time a distinct increase in the polynuclears and many of these showed karyorrhexis.

Dr. Ewing had seen a similar case before. At the autopsy foci in various stages of breakdown and softening were found in the viscera and the red marrow showed a picture resembling that seen in cases of pernicious anæmia. In other words the blood picture reflected the condition of the viscera.

Besides these two extremes I have preparations from intermediate cases. In one of these cases there was before death but little of a splenic tumor, but on the other hand much glandular infiltration. Blood smears from this case show numorous normoblasts; some of these show exquisite mitoses and the blood picture is otherwise that of a secondary anæmia of high degree. It is frequently stated that while nucleated red corpuscles are readily found in the myelogenous form they are usually rare in cases of lymphatic leukæmia. This seems to me

a mis-statement, the presence of the blasts going simply hand in hand with the nature and degree of the anæmia which may be present.

It suggests itself to me that the extent and nature of the anæmia which may be present depends upon the site and nature of the metastases, on the amount of breakdown in such foci and the consequent absorption of metabolic products from them. At all events it appears to me that the careful study of the red blood and leucocytes other than the usual lymphocytes is of paramount importance when it is a question of what progress the disease is making, especially also as there does not appear to be any constant relationship between the splenic tumor if present, or the glandular enlargement, and the progress of the disease.

Dr. F. C. Wood said that the difficulty of determining the hæmoglobin in blood containing a large number of leucocytes could be easily overcome by using the Fleisch-Mischer hæmoglobinometer, and after filling the diluting pipette with the blood and distilled water to blow out this mixture into a centrifuge tube and remove the white corpuscle by centrifuging. This would take only a few minutes, and the cells of the hæmoglobinometer could be filled with the clear supernatent fluid and an accurate determination easily carried out. If the hæmoglobin content were low, a one to one hundred mixture could be made and centrifuged, instead of the usual one to two hundred dilution used for the Fleisch-Mischer apparatus.

Dr. L. B. Goldhorn showed how he counted blood cells: The stem of the pipette is divided into ten divisions. It is customary to draw blood up to 0.5 or 1.; not infrequently one goes a little above this mark. It is not advisable to blow the blood back because one will get a somewhat higher count but there is no reason why one should not go to any one of these divisions, remembering that any division below 5 makes it somewhat more inaccurate.

Suppose one has drawn the blood up to the 8th division and has counted in the total field of 400 squares, 32 leucocytes. In order to figure out the number of leucocytes, one simply divides by the number of divisions of blood taken up in the pipette. In this case the number stated was 8; the result of dividing 32 by that is 4. On to this add three ciphers or in other words the count will be 4000. Hence in counting leucocytes draw the blood to any one of these points and divide the number of leucocytes in the total field of 400 squares by the number of divisions of blood taken and put on 000; *i. e.*, multiply by 1000. As to red corpuscles: Supposing one counts 80 squares. In these 80 there are 475 red blood corpuscles; then there are 4,750,000 per cubic millimeter for the following reason:—475 corpuscles have been counted; in order to find the number per cbmm. it is customary to find the average for one square by dividing by 80 and multiplying by 10 to get the cubic millimeter, by 400 to get total square, by 200 to make up for the dilution. $10 \times 400 \times 200 = 800{,}000$.

$$\frac{475 \times 10 \times 400 \times 200}{80} = \frac{475 \times 800{,}000}{80} = 4{,}750{,}000$$

Instead of dividing by 80 and multiplying by 800,000 one simply multiplies by 10,000 the number or corpuscles counted in 80 squares if the dilution is x 200.

Supposing the blood was drawn to a point other than the 0.5 division; then the rule is as follows: Count 80 squares; divide number of corpuscles counted by number of divisions of blood taken and multiply by 50,000.

COMPARATIVE MORPHOLOGY OF VACCINE BODIES.

JAMES EWING, M.D.

Dr. Ewing presented specimens and drawings of vaccinia of the rabbit's cornea, frog's skin and cornea, and

mud-pupy's (*necturus*) skin: diphtherial necroses of the rabbit's cornea, frog's cornea and skin and salamander's skin; and the skin of a case of measles dying in the full eruptive stage of the disease. The specimens were stained by fuchsin and indigo carmine (Borrel's method).

In describing the specimens the speaker said in taking up the study of variola in 1901, he had chiefly in mind the hope that the application of Nocht's stain to smear from the lesions of the skin and respiratory mucus membrane might be successful in demonstrating the presence of a protozoon. The results proved negative. Likewise the results of a study of fresh material examined on a warm stage, from variola and vaccinia were negative not only as to the presence of any recognizable protozoon but as well in regard to the question of amoeboid motion of any definite structures in the tissues. The histological study of the tissues while defining a great many structures which suggested a protozoon origin had left him as it had previously left the majority of observers, not only in doubt about the origin of many of the suspicious forms, but with the moral certainty that the morphological study of the vaccine body series in variola and vaccinia could hardly be relied upon to yield positive conclusions even to the most experienced observer, while the type of evidence reasonably demanded to convince others of one's conclusions regarding the nature of these bodies seemed much less likely to be obtainable from this line of study. It seemed possible, however, that the study of changes in the epithelial cells of other classes of animals which might be made to react to vaccine could yield information of value by establishing a comparative morphology of vaccine bodies. The conflicting statements regarding the possibility of producing genuine vaccine bodies by other means than by vaccine

also suggested that this subject deserved attention from the same point of view, and would especially assist in separating simple degenerative products from what might prove to be a real protozoon among the host of peculiar structures seen in variolous lesions.

With these objects in view he had compared the changes occuring in the epithelial cells in human variola with those occuring after vaccination in the rabbit, frog, mud-puppy (*necturus*), and salamander, with those resulting from the action of diphtheria toxin, after Sikowsky's suggestion, in the cornea and skin of the rabbit, frog and mud puppy, and with those seen in measles.

Among the characteristics of the ordinary vaccine body might be mentioned the perinuclear position which is not invariable, the clear zone surrounding the body which is probably an artefact but a very constant one with the larger bodies and the variable staining reaction, some bodies being basophile, others acidophile, many showing both basophile and acidophile portions. The more recently added members of the series, including signet rings, rosettes, crescents, and "encysted" bodies, must be regarded at present as of uncertain relation to the simpler bodies first described by Guarnieri.

According to his own observation the most significant feature of the ordinary vaccine body is the fact that some are basophile, some acidophile, and others possess both basophile and acidophile portions either compact or granular or vacuolated.

Proceeding to a brief description of the bodies observed apart from variola in man or vaccinia of the rabbit's cornea, might be mentioned first the bodies produced by vaccination of the frog's skin and cornea. Here he had more negative than successful results and had inoculated many frogs with any definitive effect on

the epithelial cells. In the best results a localized lesion of the cornea, and a more diffuse lesion from extensive scarification of the skin were observed. In both locations the bodies were much more numerous than in variola and included many small acidophile and basophile perinuclear forms, some rings, cysts containing 10—50 homogenous globules or vacuolated bodies, or rings. In the frog's skin the specimen exhibited seemed to show rather clearly that many of the bodies had resulted from the comminution of the nucleus. Others had a different origin which seemed explicable from the changes produced by diphtheria toxin. In the same locations in the frog diphtheria toxin in 24 to 48 hours induced cell changes and nuclear and cytoplasmic alterations which seemed to differ from those following vaccination only by their greater intensity. Mechanical influence might have been responsible for some of the changes following vaccination of the frog's skin but not in the cornea, but numerous negative results of vaccination of the frog's skin indicated that the effects of trauma were unimportant. He was inclined to believe that a true progressive vaccine process existed in the skin of the frog in the case exhibited since this lesion was so extensive.

Comparing the lesions produced by diphtheria toxin with those of vaccine in the rabbit's cornea, there appeared many points of similarity and quite as many differences. The toxin failed at least after 48 hours to cause the swelling of the cell layer characteristic of vaccine, and the lesion was more intense and the bodies more numerous than with vaccine. Among the changes produced by diphtheria toxin were (1) keratinization of the whole cells or of the whole cell layer, the nuclei staining blue after Borrel's stain; (2) keratinization of

cell body alone; (3) keratization of the perinuclear zone of cytoplasm; (4) separation in globular form of keratin masses which, either in the same or in other cells assumes the perinuclear position and appearance of the vaccine body; (5) transformation of the cell into a cyst containing a great variety of globular, or vacuolated and ring shaped bodies. Some of these were strikingly geometrical in form and might be described as rosettes. (6) A peculiar series of changes consisted in the formation in the nucleus of one or several large or minute acidophile rings. (7) From the nucleus was sometimes extruded a large strongly acidophile cyst-like body inclosing acidophile granules and minute rings. (8) The peripheral portion of many of the globular bodies broke up into granules which were often regularly arranged in a fringe about a homogeneous central body which was either acidophile or basophile.

The fate of all that portion of the nucleus except the nucleolus was a matter of great interest. Careful study of the specimens seemed to demonstrate that the nuclear material apart from the nucleolus did not become incorporated in the fuchsinophile substance but took the form of the basic staining vaccine body.

In many nuclei with or without nucleoli, there appeared a homogeneous basophile substance which sometimes showed convoluted edges, or many regularly placed vacuoles or became broken up into rings. This substance stained brown or blue with Borrel's method, and black after iron-hematoxylin. Very similar vacuolated nuclear bodies were seen in measles.

Fuchsinophile intranuclear vacuolated bodies and rings were encountered in diphtherial necroses, and in some foci the changes consisted almost exclusively in the formation of intra- and perinuclear acidophile rings. In

variola, especially in the trachea, the multiple nucleoli of the epithelial cells could often be seen to increase in size and to develop several small or large vacuoles, and to appear as congeries of rings.

In general it could be said that while diphtheria toxin does not reproduce the corneal lesion of vaccinia it produced definitely many of the typical forms of true vaccine bodies.

In the rabbit's trachea diphtheria toxin produced a great variety of spheroidal or ring shaped, or vacuolated intra-cellular bodies, many of which resembled the bodies seen in the human trachea in variola.

In the rabbit's bladder, diphtheria toxin produced the usual changes in the epithelial cells, and here many red blood cells had been extravasated into the epithelial layer, fragmented, and lodged between the epithelial cells or about their nuclei.

In the mud-puppy (*necturus*) the inoculation with vaccine in the skin and cornea produced a good deal of keratinization of cells. This formation of homogeneous acidophile material could be seen very definitely in the very large cells of this animal as beginning in the cytoplasm near the nucleus and forming an isolated perinuclear mass resembling a huge vaccine body. Yet the resemblance to the vaccine bodies of the rabbit was extremely distant on account of the large size of the cells. The nucleus and body of the pigmented cell of the skin in necturus frequently became fused into a homogeneous mass and assumed a perinuclear position in other cells. Changes in the nucleoli were not observed in necturus.

Inoculation of the skin of the salamander produced much the same lesions as in the frog, but the bodies were much larger and much difficulty was experienced in dis-

tinguishing the globules derived from epithelial cells from fragments of red blood cells.

Measles.—In the skin of a case of measles dying in the full eruptive stage of the disease, the epithelial cells in numerous marked foci and diffusely, were found to contain a great variety of intracellular bodies many of which were identical in appearance with the bodies in variola. In certain foci these bodies were much more numerous than he had ever seen in variola. They presented a greater variety of form, and the same variation between acidophile and basophile staining tendency. Keratinization of complete cells and fragmentation of these cells was frequently encountered. Indeed the whole process seemed to be a part of the hyperkeratosis which produced the large flakes thrown off in desquamation in this disease. Among the separate bodies were some which might be described as rings, basophile crescents with acidophile nuclei, vacuolated crescents, pyramidal bodies capping cell nuclei, large basophile or acidophile bodies with vacuoles symmetrically arranged, and many other forms shown in the colored sketch. He could find no satisfactory evidence that these bodies had any other origin than from the epithelial cell bodies and nuclei. After Borrel's stain, the resemblance of these bodies to those produced by diphtheria toxin, or to those seen in variola appeared to be distinct. The acidophile forms seemed traceable to nucleoli and keratinized cell bodies, and the basic bodies, blue and brown, appeared to come from the basophile elements of the nuclei. Nevertheless the resemblance of many of these bodies to developmental forms of a hypothetical protozoon was admittedly great.

The occurrence of keratinized cells in the epithelial layer had been described and depicted especially by Bosc in variola and *clavelée* (Sheep-pox), (*Cent. f. Bact., Bd.*

XXXIV, p. 413). This observer correctly stated that whole keratinized cells were readily separated from the "infected" cells in these lesions, but he did not appear to have considered the possibility that these cells might become fragmented and the fragments englobed by healthy cells. Many of the parasitic forms appearing in his sketches were definitely reproduced in the lesions caused by diphtheria toxin.

Anna Foa had recently reported (*Archives de Parasitologie, T. VII, p. 508*) that the bodies produced by diptheria toxin did not resemble true vaccine bodies which, however, she believed to be degenerative products. No details of this failure to verify Sikowsky's results were given and this part of her extensive study of vaccine bodies did not appear to have been regarded as of much importance.

Summary.—The conclusions drawn from this study were as follows: The inoculation of vaccine and diphtheria toxin in various animals produces a peculiar form of coagulation-necrosis or keratin necrosis which has been imperfectly recognized and understood, and which results in the formation of homogeneous acidophile material that may be drawn out into fibrils, or broken up into globules, or granules, vacuolated, and englobed by the more healthy epithelial cells in which it appears in the positions occupied by vaccine bodies.

This type of necrosis occurs in variola and measles and the bodies produced by it are abundantly present in the epithelial lesions of these diseases. Some of these bodies have been included in the cycles of hypothetical protozoa described by many authors in variola.

While forms of a true protozoon may be mixed up with these degenerative products in variola and measles,

he was unable to convince himself that any such theory was necessary to account for the suspicious bodies.

Some of the suspicious bodies in variola he had been unable to find fully paralleled in diphtherial necroses, especially the presence within the nuclear membrane of several distinct rings of considerable size, and he intended to repeat the systematic comparison of variolous and diphtherial lesions before concluding definitely that no protozoan could be demonstrated in variola and measles. He was strongly of the opinion, however, that the intranuclear rings were vacuolated fragments of nucleolar and nuclear material.

Dr. A. W. Williams demonstrated (1) "Vaccine Bodies" in Monkey's Skin (prepared by Dr. Flournoy); (2) Smears from Vaccine Vesicles (Wright's Stain).

Dr. Williams said that as the specimens shown by her were labeled in detail it would not be necessary for her to describe them further. She said that since she had had the privilege of examining carefully Dr. Ewing's interesting specimens of rabbits' corneas showing in the epithelial cells changes due to diphtheria toxin, she was more than ever inclined to think that most of the changes seen in the epithelial cells in the lesions of small-pox and vaccinia are due to degeneration and necrosis of the cell contents.

The two smears, one from vaccine vesicles, and the other — a control — from staphylococcus pustules of the rabbits' skin, were brought to show that both have epithelial cells containing similar vaccine-like bodies and that both have a large number of granules in and between the cells. These granules are eosinophile, basophile and

and a mixture of both. Some of them show beautiful signet ring shapes.

The granules have been studied because of the fact that from rabbits' vaccine virus filtered through two thicknesses of the finest filter paper, has been obtained an almost clear filtrate which was bacteria-free (according to the usual tests) and in which only small granules were seen, but which produced an abundant typical vaccinia when rubbed on freshly shaved rabbit's skin.

It has been impossible, however, so far, to discover characteristic granules, though many smears have been examined. Dr. Field kindly assisted in the preparation and examination of these smears.

Among various stains used, Wright's modification of Romanowski's stain was chosen for the demonstration of these smears became it had been used recently so successfully by Wright for staining the intracellular protozoan causing Delhi boil.

Wright's method was also used for the stained specimen of Trypanosoma Lewisi which was demonstrated by Dr. Williams.

Dr. Williams thought this trypanosome might be of interest because it came from one of Novy and MacNeil's test tube cultures, the first pure cultures of a protozoan which have been obtained on artificial culture media.

The specimen given to Dr. Park by Dr. Novy about two weeks ago was inoculated into the peritoneal cavity of a white rat; the rat was killed on the eleventh day following, and from its heart-blood were made the two specimens shown, one hanging drop and the other a dried film.

The trypanosoma Lewisi is a flagellate formed frequently in the blood of rats, but seldom causing the death of the animal.

This flagellate is also of interest from the fact that it is very similar to the trypanosoma Brucei, the cause of the fatal nagana or tsetse-fly disease of horses in West Africa, and the trypanosoma Evansi, the cause of surra, a similar disease met with in India.

Since 1901 a number of cases of human infection by an apparently distinct species of trypanosome have been reported.

DYSENTERY BACILLI GROUPED ACCORDING TO THEIR AGGLUTINATION WITH SERA RENDERED SPECIFIC BY THE ELIMINATION OF THE LESS SPECIFIC GROUP AGGLUTININS THROUGH ABSORPTION.

WM. H. PARK, M.D., AND KATHERINE R. COLLINS, M.D.

Dr. Park said his reason for again bringing the subject of agglutination in connection with the dysentery bacilli before the Society, was because recent results which they had obtained from the saturation with various bacilli of sera obtained from rabbits previously treated with injections of different strains of dysentery bacilli, seem to be of considerable interest. As is now generally known the true Shiga dysentery bacilli and those fermenting mannite, although in some sera appearing to agree in their agglutination, yet in others differ almost absolutely. They had previously shown that among the group of the mannite fermenting varieties there are a number of sub-groups, each sub-group containing bacilli obtained from different sources which agree in agglutina-

tion characteristics and differ from the bacilli of every other sub-group. The various dysentery bacilli seem especially rich in affinities for such agglutinins as develop through various causes in many adult animals. It is these increased "normal" agglutinins which have caused so much confusion in grouping these bacilli. The blood of a young goat or rabbit which contains no appreciable agglutinin, for any of the sub-groups, becomes after a single infection with one variety, rich in agglutinin only for the special bacillus introduced and for others of the same sub-group. Later after repeated infections the so-called "normal" agglutinins develop which affect to a greater or less degree, all varieties of dysentery bacilli. If an animal is injected frequently during a long period of time with a single strain of bacilli, it frequently happens that other distinct varieties of dysentery bacilli are agglutinated in as high dilutions as the variety used for injecting the animals. To prove that such a serum in spite of appearances contained specific agglutinin for the variety inoculated, it was determined to follow the suggestion of Castellani, who separated the common from the specific agglutinins in mixed colon and typhoid infections. It was believed that if the serum from an animal which had been repeatedly injected with one variety of dysentery bacilli was saturated with some other variety the latter might remove all agglutinins for which it had affinity and leave the agglutinins specific for the former. This result was obtained. The following tables give a few of our experiments and allow us the hope that in this way a serum truly specific in its agglutination reaction may be obtained for any of the varieties of bacteria comprising large general groups such as those to which the dysentery and colon bacilli belong. The technique was as follows: The surface of the slanted nutrient agar

contained in six tubes was inoculated with a single type of dysentery bacilli and the culture allowed to grow for 48 hours. The bacilli were then washed off into 90 c. c. of a bouillon culture of 24 hours' growth of the same bacillus. To this emulsion was added 10 c. c. of the serum from the immunized rabbit, horse or goat. This 10% solution of the serum containing enormous quantities of the bacilli was then allowed to stand for 24 hours at 37° C. The mixture of serum and bacilli was then filtered through a small Berkfeld filter. The first 50 c. c. of the filtrate was thrown away as a certain portion of any agglutinins which might be present is retained in the filter from the first portion of the filtrate. If the original serum did not agglutinate higher than 1 to 1500 the above quantity of bacilli usually exhausted all the agglutinins which had any affinity for the bacilli. The following tables show that the dysentery bacilli in our possession when tested with exhausted sera divided into three large groups and a number of individual varieties. So as not to consume too much time only sufficient data are given to establish the existence of three sub-groups members of which have been found in widely separated epidemics, the descriptions of the varieties which differed from these, but which were only met with in single cases, being left for a fuller publication.

TABLE I

STRENGTH OF AGGLUTINATIONS OF A RABBIT'S SERUM BEFORE AND AFTER INOCULATION WITH MT. VERNON MANNITE FERMENTING TYPE "B" BACILLUS AND AFTER ABSORPTION.

Cultures	Source	Isolated by	Normal Serum	After several injections	After 12 injections	After saturation w. its own culture	With Type A	With Shiga
Shiga Type	Japan	Shiga	—10	— 10	— 10	—10	— 10	— 10
	New Haven, U. S.	Duval & Vedder	—10	— 10	— 10	—10	— 10	— 10
Non-fermentmannite	Tuckahoe, U. S.	Carey	—10	— 10	— 10	—10	— 10	— 10
	Mt. Vernon	Collins	—10	— 10	— 10	—10	— 10	— 10
	Brooklyn	Collins	—10	— 10	— 10	—10	— 10	— 10
Mannite fermenting Class A	Manila	Flexner	—10	+ 20	+150	+20	— 10	+ 20
	Baltimore	Duval	—10	+ 20	+150	+20	— 10	+ 20
	N. Y. City	Wollstein	—10	+ 20	+150	+20	— 10	+ 20
	Orange	Collins	—10	+ 20	+150	+20	— 10	+ 20
	Riker's Island	Goodwin	—10	+ 20	+150	+20	— 10	+ 20
Mannite fermenting Class B	Mt. Desert, Maine	Park	—10	+200	+600	—10	+300	+400
	N. Y. City	Hiss	—10	+200	+600	—10	+300	+400
	N. Y. City	Wollstein	—10	+200	+600	—10	+300	+400
	Mt. Vernon	Collins	—10	+200	+800	—10	+300	+400
	N. Y. City	Goodwin	—10	+200	+600	—10	+300	+400
Mannite fermenting Class C	Coney Is., Br'klyn	Collins	—10	+ 10	+150	+10	+ 10	+ 20
Class D Mannite fermenting pseudo-type	N. Y. City	Salant	—10	— 10	+ 20	—10	— 10	— 10
	N. Y. City	Collins	—10	— 10	+ 20	+10	+ 10	+ 10

TABLE II

STRENGTH OF AGGLUTINATION OF THE SERUM OF A HORSE AFTER REPEATED INJECTIONS OF CULTURES OF DYSENTERY BACILLI OF TYPES SHIGA AND MANNITE FERMENTING TYPE "A" AND OF THE SAME SERUM AFTER SATURATION WITH DYSENTERY BACILLI AND OTHER BACTERIA.

Cultures	Source	Isolated by	Serum after several injec'ns	Same serum after saturation with cultures of						
				Shiga Type	Type A	Type B	Staphylo-coccus	Pyoega-neous	Typhoid	Colon
Shiga Type	Japan	Shiga	+1500	— 10	+400	+700	+150	+1000	+300	+300
	New Haven, U. S.	Duval & Vedder	+1500	— 10	+400	+700	+150	+800	+250	+300
Non-ferment-mannite	Tuckahoe, U. S.	Carey	+1500	— 10	+400	+700	+150	+700	+250	+300
	Mt. Vernon	Collins	+1500	— 10	+400	+700	+150	+800	+250	+300
	Brooklyn	Collins	+1500	— 10	+400	+700	+150	+800	+250	+300
Mannite fermenting Class A	Manila	Flexner	+1200	+400	— 10	+500	+800	+800	+300	+600
	Baltimore	Duval	+1200	+400	— 10	+500	+800	+1000	+300	+400
	N. Y. City	Wollstein	+1200	+400	— 10	+500	+800	+800	+400	+500
	Orange	Collins	+1200	+400	— 10	+500	+800	+800	+300	+400
	Riker's Island	Goodwin	+1200	+400	— 10	+500	+800	+1000	+300	+500
Mannite fermenting Class B	Mt. Desert, Maine	Park	+600	— 10	— 10	+10	+600	+600	+30	+50
	N. Y. City	Hiss	+600	— 10	— 10	— 10	+600	+600	+30	+40
	N. Y. City	Wollstein	+600	— 10	— 10	— 10	+600	+600	+30	+40
	Mt. Vernon	Collins	+600	— 10	— 10	— 10	+600	+600	+30	+40
	N. Y. City	Goodwin	+600	— 10	— 10	— 10	+600	+600	+30	+50
Mannite fermenting Class C	Coney Is., Br'klyn	Collins	+600	+20	+10	+50	+200	+30	+100	+50
Class D mannite fermenting pseudo-type	N. Y. City	Salant	+300	— 10	— 10	+50	+100	+50	+10	+20
	N. Y. City	Collins	+600	+20	— 10	+50	+100	+100	+30	+60

It will be noticed that in the rabbit serum before the injections were commenced, there was no appreciable agglutinin for any of the varieties tested. After twelve injections of cultures of a bacillus of the mannite fermenting type "Class B" agglutinins had developed for "Class B" so that a 1 to 600 dilution of the serum gave a complete agglutination, while for "Class A" of the mannite fermenting type the agglutination took place in dilutions only up to one part to 200 and for the Shiga type no development of agglutinins occurred. When this "B" serum was saturated with either Shiga or "A" bacilli at last one half of the agglutinin remained for class "B" bacilli while all the agglutinin disappeared for the others. In the second experiment given the serum of a horse was used which through injections of both Shiga bacilli and mannite fermenting class "A" bacilli had developed agglutinins for both these varieties so that dilutions of the serum up to 1–1500 gave a positive reaction. For all other varieties agglutinins were present to a less extent. These group agglutinins acted in dilutions of about 1–1600. Through the absorption of the agglutinins by either of the varieties of bacilli used in injecting the horse all the specific agglutinins for its own class were absorbed together with most of the agglutinins common to most varieties contained in the group of dysentery bacilli; but the specific agglutinins for the other class largely remained.

It will also be noticed that many varieties of bacteria not at all allied to the dysentery bacilli caused more or less absorption of these agglutinins. Others caused almost no absorption. The bacteria which removed agglutinins were all agglutinated in low dilutions by the serum used.

Discussion.

Dr. Ewing said that the examination of Dr. Park's results in the effort to separate the specific agglutinins of very closely related bacterial species, suggested that the absorption method might be employed in the separation of such related bloods as human and anthropoid monkey blood by precipitin serum. While he had succeeded with difficulty in identifying higher monkey from human blood by diluted serum, it would seem possible to distinguish more certainly between monkey and human blood or beef, sheep, and goat bloods by the use of serum which had been saturated by one of the closely related bloods. After saturation of beef-rabbit serum with sheep blood, only specific precipitin for beef blood ought to remain and none for sheep, but very little would probably be left for either.

OBSERVATIONS ON THE THREAD REACTION; ITS OCCURRENCE IN THE HUMAN BODY.

E. LIBMAN, M. D.

There have been, up to the present time, three forms of specific reactions described — the agglutination reaction, the thread reaction and the "amorphous agglutination reaction." The last named was described by Schmidt during the last year and will not be discussed here.

I wish to speak this evening on the thread reaction, partly to indicate the necessity for further work on the subject, and partly to put on record an instance of its occurrence in the human body.

The subject was first prominently brought forward by Pfaundler, although other observers had previously noted the phenomenon. Before Pfaundler, Achard showed that the blood of animals (guinea-pigs) acquired agglutinating properties against colon bacilli only with great difficulty. Archard, and Widal and Sicard, demonstrated that the reaction against a colon bacillus which had caused an infection in the human body occurred only exceptionally. Widal had shown that it is difficult to say when an agglutination reaction with a given colon bacillus is specific. Pfaundler investigated eight cases of (non-systemic) infection by colon bacilli (cases of cystitis, peritonitis, etc). In each of these he obtained a positive reaction with the serum of the case and the respective bacillus at a dilution of at least one to one hundred, if the hanging-drop preparations were observed after twenty-four hours. In the febrile cases he obtained the thread reaction; in the others, a clump reaction. The thread reaction presented the following appearance:

The organisms were arranged in long interwoven threads, the latter being generally coiled in skeins. The spaces between the skeins or threads were clear and none of the bacilli were motile. Very few individual organisms or single groups of bacilli were found in the thread labyrinths. Upon observation with higher powers the organisms in the threads looked granular; some were club-shaped. The bacteria could be demonstrated to be alive. The threads were best seen in the weakest dilution with which it was still possible to obtain a reaction. The threads usually appeared after a few hours. In most cases there was more or less agglutination at first. Pfaundler in his experiments obtained positive reactions only when the patient's blood was tested with the organ-

ism causing the infection. He claimed, therefore, that the reaction represented more than a specific reaction, namely, an individualistic one. It is to be noted that the varieties of colon bacilli isolated from the cases varied very widely.

In some unpublished experiments made by the writer in 1898 it was found that the thread reaction was frequently obtained in cases of appendicitis and peritonitis due to colon bacillus. Only colon bacilli which were closely allied in cultural characteristics were used. Fever was present in all of the cases. The general results of the observations were that the thread reactions occurring in a dilution of one to twenty, if they were complete, seemed to be specific, and no interaction between the various bacilli and a given serum was found. The experiments were too few in number, however, to prove distinctly the absence of any interaction. (In cases of proteus infection interactions were found.) in some cases agglutination reactions only were found.

Krauss and Loew obtained thread reactions with a number of organisms, with homologous and heterolougus sera (that is to say, with the serum from the case from which the organism was isolated, and from other cases) and threw doubt on the claims of Pfaundler. However, their dilutions were in the main very low and the characteristics of the bacilli used in the experiments are not detailed.

Pfaundler in a second paper attempted to show by means of animal experiments that the thread reactions obtained with a given serum and bacillus occur only as an individualistic reaction. He states that reaction is practically not as useful as the agglutination reaction, as it is inconstant.

Krauss in a second paper came to the following conclusions:

First.—That the thread reaction is a phenomenon which occurs with certain organisms under the influence of an agglutinating serum.

Sesond.—Agglutination always precedes it.

Third.—It is not as constant as agglutination.

Fourth.—In general, the same rules that apply practically for agglutination reactions apply for thread reactions, except as regards the bacterium coli.

He bases his conclusions on the occurrence of thread reactions with homologous and heterologous coli sera. He obtained the reaction with normal serum also, and says that it is therefore not specific. He concludes, however, that threads and agglutinations usually occur in specific dilutions and with the use of a homologous serum and a homologous colon bacillus. It is to be noted, however, that a study of Krauss's experiments shows that most of his tests were made with low dilutions. Only once did he obtain a thread reaction at a dilution of one to fifty with a normal horse serum and a heterologous colon bacillus. It is questionable how much value such an experiment has as regards reactions in the human body. It was only with the use of a homologous serum and a homologous colon bacillus that he obtained the thread reaction in a high dilution (one to two hundred).

In the paracolon case described by the writer two years ago, we found that the reaction of the serum with the paracolon bacillus always took the form of a thread reaction and in that case we found that the reaction was distinctly individualistic. The reactions in that case were of the chain form which I shall shortly describe. If agglutination reactions alone had been looked for, no specific reactions would have been found.

We found, several years ago, that the same serum with the same organism no longer gave the thread reaction after a number of days if the organism was transplanted daily. The length of time varied for different organisms. It seemed to us that the loss of the reaction was due to the artificial cultivation of the bacillus and not to a loss of power of the conserved serum. Further observations are needed upon this point.

Flexner, Kruse, Duval and Vedder, have noted the occurrence of the thread reaction with an organism more closely allied culturally to the typhoid bacillus than to the colon bacillus, namely, the dysentery bacillus. In their observations, however, the thread reaction was of the same significance as the agglutination reaction.

Recognizing the necessity for further investigation with regard to this subject, we have made observations in this connection whenever possible. We have found that there are two forms of thread reaction. In the first form the bacilli grow out in long, more or less convoluted threads, but still retain their bacillary form. This is the so-called "end to end" reaction. We have found this reaction occur under conditions such that we could be positive that the reaction could not be specific. At any rate, we are prepared to say that such a reaction is even less specific than the agglutination reaction.

In the other form of the thread reaction the organisms assume a coccoid form and on first viewing the hanging-drops one thinks that one is looking at chains of streptococci. This reaction, when complete, in our experience has been specific and individualistic under certain conditions. The reactions are best obtained by making an emulsion in bouillon from a culture of the organism on agar; the suspension must be a very dilute one, just enough of the culture being used to cause

a distinct cloudiness. Normal saline solution was used in some of our experiments. Sugar-bouillon cultures should never be used, as in them bacilli of the colon group are apt to take on a coccoid form.

In one case in our observations we found the thread reaction occurring in the human body. The case was one of carcinoma of the duodenum with obstruction of the common bile-duct. (The clinical features of this case are described in full by Dr. Brill in the third volume of the Mt. Sinai Hospital Reports.)

At an exploratory operation made one day before death the gall-bladder was found very much dilated, the wall thickened from chronic inflammation and, on aspirating it a fluid was obtained which was turned over to the laboratory for examination. The fluid was clear, not bile-stained, and at the bottom of the tube was a small amount of white granular matter. On examining this under the microscope we found what first appeared to be convoluted chains of streptococci. Recognizing the possibility of this really being an instance of the thread reaction occurring in the body, the sediment was stained with the Gram procedure and the organism was found decolorized. A culture made from fluid gave a pure growth of an organism resembling the dysentery bacillus morphologically and culturally. The serum of the patient gave a thread reaction (of the second form described above—the chain reaction) in a dilution of 1 to 1000. The serum when tested with colon bacilli, typhoid bacilli and paracolon bacilli gave no reaction and the sera of a number of other patients suffering from various diseases gave no reaction with the bacillus. *On staining the sediment found in the gall-bladder with the Neisser stain, it was found that the bodies which were seen which looked like cocci were the polar bodies of the bacilli.*

It has been shown by a number of authors that the colon bacillus can, under various conditions, assume the coccoid form. Livingood found that when the colon bacillus was inoculated upon media containing unheated liver juice oval forms developed. Rodet found such forms in cultures of the colon bacillus in lactose-bouillon and very often in old cultures of the colon bacillus. He also found the coccoid form in fluid obtained from diseased gall-bladders. Dunbar also noted chain formation of the colon bacillus.

In a very important paper published by Adami, Nicholson and Abbott, a number of very interesting observations on the coccoid form of the colon bacillus are recorded. They succeeded in obtaining coccoid forms of the colon bacillus by the use of acid media, by inoculation into animals, and they describe the occurrence of coccoid forms in bile, ascitic fluid and in the livers of man and a number of animals, both normal and diseased.

Ohlmacher has described a very interesting observation in this connection: In a case of gangrenous cholecystitis with cholangitis due to biliary calculi, the colon bacillus was isolated from the gall-bladder, liver and other organs. In the first generation on agar plates very remarkable forms of the organism were found and it was only after three or four transplantations through bouillon that the usual form of the colon bacillus was found. The most marked peculiarities of form were found in the original smears from the gall-bladder and bile-ducts—all gradations from minute coccoid or diplococcoid to long, coarse filamentous forms were observed. Ohlmacher states that they corresponded precisely to those described by Adami, Abbott and Nicholson. Threads were found, some of which were quite thick, others were thin and delicate, some being quite shadowy. Some of the shorter

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ones showed a row of deeply staining inclusions and others still had clear unstainable spaces or areas with metaehromatic staining. "These filaments were often aggregated into groups, several times appearing as a mass of tangled threads." Some of the shorter rods showed short true branches. Some of the filaments or longer rods had median swellings, others among the shorter rods had clubbed ends and many of them were bent at various angles. Ohlmacher was of the opinion that the polymorphism of the bacillus was to be ascribed to certain environmental influences resulting from its abode in the biliary apparatus of the human host.

The observations made in the case which I have described cannot, I believe, be traced to purely accidental influences, nor do I believe that they were due to the icteric condition of the serum, for in the gall-bladder itself the fluid was perfectly clear and not bile-stained, and in the second place the serum did not produce such changes in cultures of other closely related bacillary forms. The reaction must be regarded as a specific one due to the inflammation of the gall-bladder set up by the bacillus in question.

The observations recorded here are of a certain degree of importance because they indicate that we must be very careful in making a diagnosis of streptococci in hanging-drops; we must remember that we may be dealing with the coccoid forms of bacilli. Thus, I have found, in a number of instances, in the urine, long chains of what appeared to be streptococci, but on careful examination these have been found to be the coccoid forms of colon bacilli or other bacilli. In these cases it is probable that the acidity of the urine may have had much to do with the development of the coccoid forms. The difficulty can be avoided, at least to a great extent, by

the use of the Gram stain, because up to the present time the thread reaction has been particularly noted with organisms that react negatively to the Gram procedure. In applying the Gram stain to urinary sediments it is important to remember that with very acid urines organisms which are naturally Gram-positive can very easily be decolorized.

Dr. W. B. Noyes asked if Dr. Libman had noted any irregularity in the staining of organisms with Gram, especially in urinary deposits.

Dr. Libman replied that he had found, as Dr. Noyes had, that great care is necessary in staining urinary sediments for the gonococcus, for in urines that are very acid. Gram-positive organisms may be easily decolorized and in urines which are very albuminous Gram-negative organisms are apt to hold the Gram stain especially if the cover glasses are overheated.

Dr. Park said in the work on dysentery at the Health Board Laboratory thread reaction occurs frequently although the forms of thread reaction spoken of by Dr. Libman as being special, are not met with in the cultures there. The other form, which the speaker thinks not specific, is met with, constantly. We must have growth of the bacilli to have reaction. A hanging-drop or tube without growth cannot have reaction. By changing temperature and nutriment in the medium we could make or not make thread reaction.

Necessarily the serum in which the thread reaction occurs would have to be specific, as in ordinary agglutination. Dr. Park thought the forms described by Dr. Libman about the same thing as in ordinary agglutination depending on temperature and the fluid it was in.

Literature.

1. Pfaundler: *Centralblatt für Bakteriologie.* 1898, Vol. 23.
2. Krauss and Loew: *Wiener klin. Wochenschrift.* 1899, p. 95.
3. Pfaundler: *Ibidem.* 1899, p. 342.
4. Krauss: *Ibidem.* 1899, No. 29, p. 761.
5. Flexner: *Centralblatt für Bakteriologie.* Vol. XXX.
6. Duval and Vedder: *Journal of Experimental Medicine.* Vol. VI, No. 2.
7. Libman: *Journal of Medical Research.* Vol. VIII, p. 168.
8. Livingood: *Centralblatt für Bakteriologie.* Vol. XXIII, p. 980.
9. Rodet: *Archiv de physiologie norm. et patholog.* 1896, p. 968.
10. Dunbar: *Zeitschrift für Hygiene.* 1892, p. 485.
11. Adami, Nicholson and Abbott: *Journal of Experimental Medicine.* Vol. IV, p. 349.
12. Ohlmacher: *Journal of Medical Research.* Vol. II, p. 128.
13. Schmidt: *Wiener klinische Wochenschrift.* 1903, p. 873.
14. McWeeny: *British Medical Journal.* Sept. 3, 1898, p. 592. A note in the thread reaction with the typhoid bacillus.
15. Of interest in this connection is the paper by Adami on Latent Infection. *Journal of American Medical Association.* Dec. 16, 1899.

NEURO-FIBROMA OF HAND.

D. S. D. JESSUP, M.D.

Dr. Jessup presented a neuro-fibroma removed from the hand of a woman in April, 1903. The patient was thirty-four years old. Two months previously her attention had been called to a swelling in the palm of her hand. This had increased in size and become painful and she came to the Vanderbilt Clinic for relief. Examination showed an elongated swelling on the palmar surface of the left hand extending from the base of the

second metacarpal bone to the middle of the first phalanx of the index finger. It lay over the flexor tendons of the index finger, was soft, apparently fluctuating, and a diagnosis of tubercular teno-synovitis was made. Under ether anæsthesia an incision was made the length of the growth and it was then easily dissected away from the surrounding tissues. The tendons were not exposed, but at the ends of the tumor a pedicle was found which proved to be a division of the median nerve. On reaching the tumor the nerve broke up into small filaments which were distributed over its surface. After removal the growth measured nine cm. in length, was lobulated, and was two cm. in diameter at its thickest points (see photograph.) The consistency was soft. Section after hardening with formalin showed firm white fibrous tissue. Transverse sections were stained by the hæmatoxylin-eosin, picro-acid-fuchsin, and Weigert methods. They showed a growth of fibrillated connective tissue which was very cellular. The whole tumor was made up of this tissue with the exception of a few bundles of nerve fibers lying on the surface, which correspond to the nerve filaments noted at the time of the operation. The tumor had apparently sprung from the perineurium and there was no proliferation of the nerve fibers. The patient did not show tumors of any other nerves, and the case was also interesting from the standpoint of surgical diagnosis.

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Dr. Otto H. Schultze, *President.*

A CASE OF HEMORRHAGIC MENINGOENCEPHALITIS. (1)

M. G. SCHLAPP, M.D.

Dr. Schlapp presented a specimen taken from a case of hemorrhagic meningoencephalitis in a child sixteen months old. The child had been well until the twenty-fifth of December, when it developed a cough, an acute foul smelling rhinitis lasting four or five days. At the end of this period convulsions appeared on the left side, involving the left leg, arm and face, later on spreading to the right side. It had previously been noticed by the mother that the fontanelles had bulged very much. The child had edema, most marked about the eyes, nose and forehead. This lasted only for about an hour and then disappeared. The convulsions grew worse and the mother took the child to a hospital where the fontanelles were punctured, showing a collection of fluid, but no change of anything else was noted. As the child grew worse, clonic and tonic convulsions developed. The child practically remained in a tonic convulsion. Acute hydrocephalus was diagnosed. About one and one-half hours after puncturing the fontanelles, the child died.

The autopsy was performed by Dr. Schultze, who was allowed only to open the cerebral cavity. He took out the brain and found a hemorrhagic area extending from the frontal pole to the precuneus on both medial surfaces, and laterally the hemorrhage extended about one and one-half inches from longitudinal fissure. This hemorrhage discolored the brain. In the longitudinal sinus was found a thrombus. It did not fill out the sinus completely. Most of the veins extending laterally from the longitudinal sinus were thrombosed. This condition

(1) Received for publication May 3, 1904.

on section looked exactly like cases described as non-purulent hemorrhagic encephalitis. The hemorrhage was confined to the cortex of the brain, giving the peculiar dotted appearance of the non-purulent hemorrhagic encephalitis. It could be mistaken for this type of encephalitis. The thrombus of the longitudinal sinus most likely caused the condition and was probably an infectious one. Besides hemorrhage and thrombosis there was a meningitis extending over the greater area of the brain. It was not confined to the hemorrhagic area, but extended to apparently normal areas. This meningitis was of the cellular type made up of round cell infiltration, mononuclear leucocytes, not pus cells. It was likely that the meningitis was caused by the same condition that caused the thrombus, or the thrombus was secondary to the meningitis. It was possible that this infection might have passed through the lymph spaces, Pacchionian bodies, into the longitudinal sinus, in that way producing thrombus. It might have been produced through the blood vessel in the same way the meningitis was produced. Round cell infiltration around the blood vessels and meninges was well shown.

Dr. Schlapp also showed a specimen from a case of hemorrhagic myelitis in a man, forty-eight years old, who two years previous to this attack of myelitis had had an attack of malaria. History of syphilis, alcoholism, etc., negative. About two months before this trouble came on he had an orchitis from a trauma. The testicle was very much swollen. It was removed by a surgeon in California. The surgeon had told him that the nature of the lesion was tubercular. The wound healed and he felt fairly well after the operation. He took a trip to the mountains where he strained himself walking and driving over rough roads, and after a particularly long drive

he had chills and felt weak in the legs. Then numbness was felt in the soles of the feet and gluteal region. The numbness disappeared and the man came East. While here the numbness again came on and in two or three days it extended from the plantar surface of the feet up to the medial part of the thigh. The gluteal region was also involved. When seen by Dr. Schlapp the man had no definite objective symptoms, although at that time it was suspicious of beginning myelitis. There was no temperature and the pulse was regular—80 to 84. The man was put to bed and watched. The day after Dr. Schlapp had seen him, he developed a subnormal temperature—97 to 96.8. This kept up for several days. In the meantime the numbness grew worse and objective symptoms appeared; there was sensory disturbance, marked pain, and temperature, sense disturbed and ataxia; later complete anesthesia below the knees, spreading up the legs and body to the region of the navel; remained one or two days in this condition and then spread to the arms and the man died of a condition resembling paralysis of the vagus and respiratory and heart centers. One and one-half days before death he developed a temperature of 103°; before that it had been subnormal. On autopsy, Dr. Elser found cirrhosis of the liver and pneumonia, which had existed several days before his death. He had had also acute parenchymatous nephritis. The bones of the spinal column were normal; no infectious condition from that source. No meningitis, no disturbance of the pelvic organs. The spinal cord from the second lumbar segment to the fourth cervical presented small hemorrhages throughout all the tissue; white and gray matter was affected alike. The area of hemorrhage varied, in some places more and in other less marked, but distinctly involving the tissue of the cord. At that time the case

was diagnosed (and subsequent histological examination verified this conclusion) as non-purulent hemorrhagic myelitis. No meningitis. These hemorrhagic conditions of the cord, Dr. Schlapp said, were not very common and the question arose, what was their cause? Cultures were made but they were negative. A Gram stain made of a section was also negative. Nothing definite was found in the section.

A drawing of a section of the cord was shown.

Discussion.

Dr. Noyes said that the first case would be extremely interesting if a report of the cerebrospinal fluid or blood from the ventricle had been made. He understood that this had not been done. In a case reported to the Neurological Society, Dr. Brooks had spoken of the difficulties of diagnosis of hemorrhagic meningoencephalitis, and careful examination of the cerebrospinal fluid sometimes facilitated this diagnosis. Much careful work had been done in the City on the cytology of the cerebrospinal fluid during the last few months. Recent articles in some French journals report that in almost all hemorrhagic conditions, such as hemorrhage into the ventricle, and hemorrhagic encephalitis, the cerebrospinal fluid showed a reddish discoloration of the serum or red corpuscles; and this phenomenon frequently makes a positive differential diagnosis possible. Dr. Schlapp had spoken of infiltration of mononuclear leucocytes of the tissues. It was interesting to compare the presence of lymphocytes in the cerebrospinal fluid and blood with the presence of the same type of cells in the tissues, lymphocytes in the fluids being chiefly found in certain classes of cases, as syphilis or tuberculosis, while

polynuclear cells indicate acute infections. Whether this coincided with what could be demonstrated with sections, or how far it occurred with types of leucocytosis in general, Dr. Noyes did not know. The diagnosis of meningoencephalitis though rather difficult, could be made by excluding the more common general and local lesions of the brain. It was sometimes found at autopsy where other diagnoses had been made.

In regard to Dr. Schlapp's second case, Dr. Noyes said it was extremely interesting because so many of those transient cord conditions are met with in cases where paralyses have come and disappeared, and one can only infer that some vascular lesions have occurred. Possibly the vessels may have been obliterated but the changes may not have gone so far as the cases Dr. Schlapp had shown. Probably lesions of this type occur more frequently than is supposed.

Dr. Schultze said the cases were also interesting from a medico-legal standpoint. As far as his own experience went he thought the occurrence of a true hemorrhagic type inflammation rare. The cases which Dr. Schlapp had described might readily be mistaken for cases of trauma unless that condition were kept in mind. It was possible for an inflammation of the pia and the brain or cord, or for a thrombosis of the longitudinal sinus, to produce a condition which might be mistaken for laceration of tissue with subsequent hemorrhage.

Dr. Schlapp said that he considered the remarks made by Dr. Noyes as important as to lumbar puncture in the second case. In the first case he thought diagnosis would have been made before if the physician had taken the mother's report. She said the child developed convulsions on one side, then on both. The child had edema of the tissues of the frontal bone over the eyes

and eyelids. This, he thought, would have lead the physician to think of thrombus of the longitudinal sinus at once. If the eyes had been examined a protusion of the central vessels of the eyes would have been observed.

TERATOMA OF THE BROAD LIGAMENT.

FERDINAND M. JEFFRIES, M.D.

Dr. Jeffries said, in presenting a teratoma of the broad ligament, that he thought the specimen of more than usual interest. He thought it unique in character. It was taken from a girl of seventeen—a virgin. The history dated merely two days previous to the operation, although since the operation when the growth was removed, the surgeon had gleaned the fact that the girl had been troubled for some time with constipation and that she was very easily fatigued, and on some occasions suffered considerable pain which was supposed to be in some way connected with the constipation.

Two days previous to the operation the girl consulted her family physician, but it was merely for constipation. He gave her some laxative. Next day he called, and found the patient in distress with no action of the bowels, and upon examination found an enlargement of the left inguinal region, which he had not seen at the first examination. He did some manipulation and increased the laxative dose. The next day he was called in great haste and told that quite an enlargement was noticeable in the left inguinal region. Then he called in a surgeon. There had been, up to that time, no action of the bowels. The surgeon was at a loss to know what it was, but there had been a state of collapse and tem-

perature previous to his arrival. The surgeon advised operation, and he went in through the cul de sac. As soon as he made an incision he found a hematoma. He got rid of the clot of blood and in his search found what appeared to be membrane, and made up his mind that there was pus. He made an incision through the membrane, but instead of pus coming, there was a sero-sanguinous fluid containing a lot of fat, partially saponified or emulsified. He traced this along and came to the growth shown by the speaker. Although found in the left inguinal region, it was attached to the right broad ligament. The surgeon took the entire growth out through the cul de sac of Douglas. It was a cyst about the size of a child's head.

It was a body covered with skin, having peculiar toe-like enlargements, some large, others small. There was floating with the fat a quantity of hair.

The cyst contained bone and cartilage, the one described as toe-like is a picture of the tarsus bone. Some of these bones were harder and less cartilaginous than others. No histological examination had been made.

Dr. Jeffries also showed a cast of the bronchial tubes, a regular tree from a case of plastic bronchitis, more often seen in pictures than in reality.

He also showed a sample of urine of unusual interest. It had been put under the microscope. It was urine filled with ameba. At first it was thought that they might be due to water used to wash the bottle in which the urine was placed, or perhaps the centrifuge or pipette. In this urine in every field, the speaker had seen with the one-sixth objective contained seven or eight amebæ, some exceedingly large, one or two half filling the diameter of the one-sixth objective.

The patient was a young man, about thirty-five years

of age, who had measles. He had a stricture and his urine contained pus, and apparently had done so for a considerable period of time previous to the present attack of measles.

The sample was presented for enlightenment. It was conceivable that there might, in some cases of dysentery, be new channels opened up from the bladder and amebæ coli might enter, but he thought these were proteus ameba—perhaps there was no difference except in the locality where found. These ameba did not seem to contain blood cells. He was not prepared to state what bodies were contained. Dr. Jeffries had that day been able to find ameba in a case of amebic dysentery where he could show blood corpuscles. These did not look like chlorophile bearing bodies.

Discussion.

Dr. Larkin asked Dr. Jeffries, in regard to the specimen of urine, if he had examined the ameba when warm; and also if he had noticed the peculiar actions of the ameba one is liable to get in warm urine.

Dr. Jeffries replied that he got the urine fresh and examined it in half an hour after it was passed. He had noted motility and all.

Dr. Field asked what was the nationality of the patient and whether Dr. Jeffries had at any time found red blood cells in the ameba.

Dr. Jeffries replied that the man was an American and had never been out of this country. He had not at any time found red blood cells in the ameba.

THE BACTERIOLOGY OF THE BLOOD IN TYPHOID FEVER; A REPORT ON SIXTY CASES, AND A STATISTICAL STUDY.

WARREN COLEMAN, M.D., AND B. H. BUXTON, M.D.

The authors made bacteriological examinations of the blood in sixty cases of typhoid fever in Bellevue Hospital, and have collected five hundred and forty-four similar cases from the literature, giving a total of six hundred and four cases. Their practice was to distribute ten c.c. of blood taken from one of the large veins at the elbow, into four flasks each containing one hundred c.c. of broth and to incubate at 37° C. When the cultures were positive they tested the bacilli in glucose gelatine, litmus milk, neutral red agar, and other differentiating media. They were also tested for their agglutinating properties in an active serum. A reaction at 1:100 was considered sufficient for diagnostic purposes.

Believing that paratyphoid infections should be considered clinically as cases of typhoid fever, they have included in their totals the twenty-three cases of so-called paratyphoid fever in which bacilli have been found in the blood.

Seventy-five per cent. of the six hundred and four cases showed the presence of bacilli in the blood at some stage of the disease and the authors felt inclined to regard even this as somewhat lower than may be expected in future investigations, since the most recent records, those of Busquet and of Courmont and Tesien, show that with the most improved methods the bacilli are practically uniformly present in the first three weeks of the disease.

Analyzed by the week in which the examination was made it was found that:—

1.—Of eighty-five cases in the *first week* positive results were obtained in seventy-nine or 93%.

2.—Of one hundred and ninety-eight cases in the *second week* positive results were obtained in one hundred and fifty-one or 76%.

3.—Of one hundred and fifteen cases in the *third week*, positive results were obtained in sixty-five or 56%.

4.—Of fifty-five cases in the *fourth week*, positive results were obtained in eighteen or 32.7%.

The striking fact brought out by this analysis was the large percentage of positive results obtained in the first week of typhoid fever with the steady decline thereafter. From this it appears that the earliest and principal seat of infection in typhoid fever is the blood. The authors believe that the bacillus disappears from the blood about the end of the third week in the ordinary case of typhoid fever, and that its failure to disappear at this time means the prolongation of the clinical course of the disease.

Twenty-one cases were examined during a relapse, and the bacillus was found in all but three of them.

Ten per cent. of the six hundred and four cases presented the bacillus in the blood before the Widal reaction could be obtained. The Widal reaction is often delayed to the twentieth to the thirtieth day; but in one of their cases did not appear until the seventy-first day; hence it was felt that the absense of this reaction by no means justifies the diagnosis of paratyphoid infection.

Two of the authors' cases gave the bacillus coli communis in pure culture in the blood. Both cases were mild and the question was raised whether the colon bacillus might cause a primary systemic infection.

Discussion.

Dr. Gilman Thompson said he thought Dr. Coleman and Dr. Buxton were to be congratulated for the work they done on this timely subject. Upon the practical side of the results, he wanted to say a word. An absolutely accurate test would be of great value in an early diagnosis of typhoid fever, and also in those cases in which one may be in doubt later in the disease as between the existence of typhoid fever and some of the conditions for which it is occasionally mistaken, like an obscure case of malignant endocarditis. in regard to the bacteriological examination being of use in very early diagnosis, he asked Dr. Coleman how soon he could guarantee a positive result, if such existed, after obtaining a specimen of the blood.

Dr. Coleman replied in about three days.

Dr. Thompson said that that reduced the value of the result for early diagnosis. If the specimen were obtained on the fourth day, and three or four more days elapsed before results were obtained, the test did not improve on the Widal reaction which was often apparent by the end of the first week, or upon the appearance of eruption, etc. In that respect he thought the test a disappointment as an early one to be used as a help in the matter of diagnosis at the bedside.

The speaker had, however, had a number of cases examined at the Presbyterian Hospital, positively demonstrating the presence of bacilli before the Widal reaction was obtained. The Widal reaction had been behaving in a peculiar way. When the test was first used in the city, the speaker was interested in collecting data. He gathered data from other clinicians to the number of five hundred cases. In 93% of these cases the Widal reac-

tion was found during the active course of the disease, a large percentage being at the end of the first week. With recent tests the reaction was absent in many cases typical, clinically, of typhoid fever, which tests are not returned as positive either from the Health Board or specially trained observers in the laboratory even after three or four weeks, or perhaps when the patients are in convalescence or relapse. The technic of the Widal test had improved and examiners were reluctant to report a positive reaction until it was obtainable with a much greater degree of dilution than that at first employed. Dr. Thompson believed that bacteriological reaction of the blood could yet be made more satisfactory, and used as an earlier test then the Widal reaction.

The reference of Dr. Coleman to the new aspect in which typhoid fever is regarded; that is, as a blood toxemia, was very interesting, for it afforded an explanation of certain phenomena of the disease, particularly such complications as involved the gall-bladder, periostitis of the rib, phlebitis, etc.

In regard to the paratyphoid germ, demonstration of its presence in the blood was not as yet of any real clinical value in distinguishing the varieties of typhoid fever at the bedside. Paratyphoid had been reported to him by bacteriologists, when he had been entirely unconscious from the clinical standpoint that he was dealing with anything but ordinary typhoid fever. The bacilli of the ordinary typhoid had been found in mild and severe cases, and so it seemed in regard to paratyphoid bacilli. Five cases of apparent typhoid infection had been brought to the Presbyterian Hospital from a tenement house. They were thoroughly investigated by the Health Board from an etiological standpoint; and one which came to autopsy appeared to be typhoid fever, but

paratyphoid was reported in two or three of the cases. These cases did not present any clinical peculiarities. This recalls the difficulty in differentiating degrees of malignancy in types of diphtheria and tuberculosis germs. The typhoid bacteriology is interesting in connection with the study of the natural history, in general, of these germs. Dr. Coleman had emphasized the futility of the use of intestinal antiseptics in typhoid fever. The speaker thought the discovery of these bacteriological tests ought to put a final quietus on the attempts to treat typhoid fever with intestinal antiseptics alone. No better proof of this was needed than that brought out by such research as that under discussion.

Dr. Norris asked how many cases of typhoid fever Dr. Coleman had found in which he could not obtain the Widal reaction, and in how many cases he found concurrent infection with typhoid.

It was rather hard to draw conclusions from broth cultures because in that medium at no time could the original number of bacilli found in the blood be estimated. It was a favorable medium for the isolation of typhoid bacilli, however.

Dr. Field said that he objected to their considering a disease as a septicemia because micro-organisms were found in the blood. Dr. Libman had shown that numerous surgical cases suffering from localized staphylococcus aureus infections showed many staphylococci in the blood. He thought that they were absorbed from the point of infection and that they did not increase or multiply in the blood stream. In typhoid fever, he said, there were a great number of typhoid bacilli in the intestine and it was perfectly conceivable that these organisms could find their way into the blood stream by way of the lactals,

especially when there was food undergoing digestion. Where the number of typhoid bacilli per c.c. had been determined they had always been found to be few. In those cases of typhoid fever which have been called typhoid septicemia, the bacilli were present in large numbers per c.c. and the clinical picture was entirely different. The speaker did not think it proper, therefore, to term the disease as ordinarily seen, a septicemia because a few bacteris were found constantly or in a large proportion of the cases in the blood stream. He did not think that it was in the blood stream that they proliferated and caused that profound toxemia seen in this condition.

Dr. Libman said that he agreed with Dr. Field that typhoid fever should not be considered as a general disease simply because some bacilli were found in the blood. It was different with cases in which there was no local lesions. To illustrate the point that bacteria might be in the blood, even in large numbers, without setting up any secondary foci or seeming to make much trouble, he referred to a case of osteomyelitis of the humerus which he had recently seen. The pus from the bone contained the staphylococcus aureus in pure culture and by means of a blood culture it was demonstrated that there were five hundred colonies of the staphylococcus aureus to each c.c. of blood. After the focus in the bone was opened up the temperature dropped considerably and four days after the operation the blood was found sterile and the patient recovered.

Dr. Libman also thought that it was hardly conceivable that the symptoms of typhoid fever, as a rule, were due to the presence of the bacteria in the blood because so few of them were usually found. He believed that it might be possible to obtain results earlier if inoculations

were made from the flasks containing the blood even before any turbidity developed from the growth of bacteria.

With the Widal reaction his results had differed from those of Dr. Thompson. The results had been better than those in previous years. During September, October and November, every case of typhoid had given a positive Widal reaction within three or four days after admission.

He believed that the finding of the typhoid bacillus was of importance in establishing the etiology of otherwise obscure conditions. There had been admitted into the service of Dr. Rudisch, at the Mt. Sinai Hospital, a patient suffering for twenty-four hours from symptoms apparently due to intestinal perforation. On operation a localized pus sac was found, a culture from which showed staphylococcus aureus only. The were no typhoid bacilli in the urine or stools, but the blood, two days after admission, was proven by culture to contain typhoid bacilli. The Widal reaction became positive about a week later. In that case the diagnosis would not have been definitely established so early without the blood culture.

Dr. Coleman said that only one or two of the authors consulted made mention of mixed infections in typhoid fever. In a few cases Busquet had found other organisms than the typhoid bacillus; e. g., streptococci, staphylococci, and pneumococci.

Dr. Buxton said he had found no evidence of mixed infections in the cultures.

Dr. Coleman agreed with Dr. Field that typhoid septicemia was not a good term, and said that they had used it only to emphasize the fact that typhoid fever was not an intestinal disease, susceptible of modification by means of intestinal antiseptics.

DEMONSTRATION OF COAGULUM PRODUCTION BY GROWTH OF BACTERIA IN SUGAR MEDIA.

E. LIBMAN, M.D.

In a paper published several years ago on the features of the growth of bacteria in media containing both sugars and serum, I mentioned that I had occasionally seen coagulum production in such media and suggested that the phenomenon might become of some importance in explaining the occurrence of some cases of thrombosis in infections, especially in diabetes.

Since that time Dr. Flexner had brought up the question of the agglutination of red blood-cells as a cause of thrombosis.

The flask which I present contains streptococci growing in a medium containing ½% glucose-bouillon two parts and serum one part. In it can be very nicely seen the very extensive coagulation and at the bottom of the flask marked agglutination of the erythrocytes. Central flasks containing no cocci showed no changes after several days.

SOME PECULIAR CHANGES IN THE FLUID OF A PLEURAL EFFUSION.

E. LIBMAN, M.D.

This fluid when first obtained by aspiration of a chest in a case of pneumonia was light yellowish in color and very slightly turbid. Microscopically it contained very few pus cells and some pneumococci. On standing in the thermostat twenty-four hours a precipitate began

to form in the tube and the fluid turned greenish in color. After forty-eight hours the fluid was decidedly green, no longer transparent and there was a heavy clumpy deposit at the bottom of the tube. Without a microscopic examination one would have believed that the fluid came from a case of empyema.

The specimen is shown merely to indicate that certain appearances which we see in some exudates can develop as a result of bacterial growth outside of the body.

It has been observed that certain streptococci can produce a greenish color in media which contain a decided amount of blood and we occasionally see fluid obtained from the cerebrospinal canal turn slightly green after standing a few days, but the marked change which I have described from a practically clear fluid to a fluid resembling closely an empyema fluid, has, I think, not been noted.

PERIOD OF THE GREATEST ACCUMULATION OF TETANUS TOXIN IN BROTH CULTURES.

CYRUS W. FIELD, M.D.

Dr. Field read a paper on his experiments on this subject. In preparing toxin for the purpose of immunizing horses to tetanus, he said that it was important to obtain as strong a toxin as possible, as there was then less volume of bouillon to inject. In preparing toxin for this purpose at the Research Laboratory of the Department of Health, it was the custom to grow the organism for nine or ten days in flasks holding about two liters of bouillon which had twelve c.c. of normal NaOH solution

added to it after having been made neutral to litmus. The bouillon was covered with a thick layer of paraffine about one-half to three-quarters of an inch thick. This sealed it off and gave a practically anærobic condition. For every liter of bouillon about twenty c.c. of an old tetanus bouillon culture was added before the paraffine had hardened. The strength of this routine toxin was found to vary greatly, one week a one-thousandth of a c.c. being the minimum lethal dose; perhaps the next a fiftieth or even a tenth. Thinking that perhaps it was the length of time for which the culture was grown, Dr. Field was determined to take a sample of toxin from the flask each day and test its toxicity. For this purpose, alboline, which is a liquid paraffine, was used instead of paraffine to seal off the media. This permitted the introduction of a pipette and the drawing off of any amount of culture without disturbing its anærobic condition. A sample of the culture was removed each day at the same hour, a portion being titrated to determine the degree of acidity and the rest passed through a Berkefeld filter; a portion of this filtrate was also titrated and the rest was used to make up the various dilutions for injection. Tetanus toxin is such an unstable compound that one has to determine its minimum lethal dose by making all the possible dilutions and injecting them at the same time, as the following instance will show. Having filtered two liters of a culture, Dr. Field tested it and found that its minimum lethal dose at that time was $\frac{1}{500}$ of a c.c. The next day, it being needed for purposes of immunization, it was again tested before being sent to the veterinarian, and at this time the minimum lethal dose was $\frac{1}{50}$ of a c.c. The titration in all this work was done with a twentieth normal NaOH solution, phenolphthalein being the indicator. The method of

determining the minimum lethal dose was as follows: The filtered bouillon was added to the sterile water in various dilutions, for instance, on the first day five dilutions were made up, one c.c. of each containing respectively $\frac{1}{10}$, $\frac{1}{25}$, $\frac{1}{50}$, $\frac{1}{100}$, and $\frac{1}{250}$ of a c.c. of the bouillon culture. One c.c. of a dilution was injected into the muscles of the hind leg of a three hundred gram guinea-pig. If the pig died in four days with symptoms of tetanus poisoning he was considered to have received a fatal dose of toxin, so that the pig dying from the effect of the highest dilution was considered to indicate the minimal lethal dose of that toxin. As will be seen it required a large number of guinea-pigs to carry out the testing of a culture in this manner.

The acidity of the unfiltered sample was always much higher than that which had been drawn through the Berkefeld filter, Dr. Field's explanation for this being that the bouillon which had not been filtered contained a great amount of sulphureted hydrogen which is one of the gases formed by the tetanus bacillus in its growth. This gas is acid in its reaction. When the bouillon was drawn through the Berkefeld filter it entered a vessel from which most of the air had been exhausted, and therefore any gases which might be held in solution were rapidly drawn out of the bouillon. When this gas is sulphureted hydrogen, giving the bouillon an acid, reaction, there is naturally a great lessening in the degree of the acidity after it had passed out. To determine whether or not this explanation would hold, Dr. Field took some water which contained large quantities of sulphureted hydrogen and determined its degree of acidity. It was found to require three c.c. of a twentieth normal NaOH solution to neutralize it. This was passed through a Berkefeld filter into a vessel in which the air had been

exhausted, and was allowed to remain there five minutes. It was again withdrawn and its acidity determined. This time only 2.2 c.c. of the same solution were required to neutralize it. Tetanus toxin remained in a vacuum much longer, as a rule, than five minutes, so it would seem that the high acidity in the unfiltered sample was due to the bouillon containing a large quantity of sulphureted hydrogen in solution.

Dr. Field's conclusions drawn from this work, were as follows:

1st.—On the average the best results will be obtained if the culture be grown only six or seven days in the incubator and after filtration be kept in the ice box.

2nd.—No toxin should be used after fourteen days have elapsed from the date of inoculation.

3rd.—As a rule, the degree of acidity of the unfiltered bouillon varies directly as the amount of toxin formed, but sufficient work has not been done to permit any definite statements.

Dr. Field said that at some future time he intended to test the effect of the toxin before filtration to see if it too was affected in any manner by the vacuum and also to test the power of an old reduced toxin to neutralize tetanus toxin.

CALCIFIED THROMBUS OF THE INFERIOR VENA CAVA.

GEORGE P. BIGGS, M.D.

Dr. Biggs presented a specimen. He said the patient from whom the specimen was obtained was unconscious when admitted to the Hospital, and died three days later without giving any history. He was a well-

nourished man, an engineer, aged sixty-one years, a native of the United States.

At post-mortem the immediate cause of death was found to be fracture of the skull with extensive cerebral laceration. The vessels at the base of the brain were very sclerotic. Fibrous puritic adhesions were numerous. Left ventricle of the heart hypertrophied. Valves normal in structure; but there was relative aortic insufficiency. The entire arch of the aorta was uniformly dilated to a diameter of about five cm. and its wall very much thickened, measuring six to ten mm. The enlarged arch made moderate pressure on the right auricle, superior vena cava, trachea, and pulmonary artery. Sclerotic changes were marked throughout the aorta and there were numerous areas of calcification.

Spleen was normal in size and consistency, but firmly adherent to surrounding organs. Right kidney larger than normal, left slightly smaller than normal; both presenting similar lesions aside from variation in size; viz.: moderate chronic nephritis with numerous small cicatrices.

Microscopic examination showed moderate amyloid degeneration of kidneys and spleen. Liver normal in size and general appearance except for a few small cicatrices. The foregoing lesions are mentioned chiefly because of their strikingly syphilitic nature.

The nature of the specimen demonstrated by the speaker was not appreciated until after its removal from the body, and changes in the circulation could not be accurately determined. Distinct enlargement was, however, noted, in the azygos, iliac and renal veins.

The inferior vena cava from its beginning up to the level of the renal veins was completely occluded constituting a hard, absolutely rigid column firmly adherent to

the aorta. The diameter of the vena cava is one and one-half to one and three-quarters cm. In the center of it is a small, calcareous rod-like portion, one-half to three-quarters cm. in diameter, extending throughout. The balance of the structure is chiefly fibrous tissue, all traces of the thombus having been replaced thereby. One naturally thinks of the syphilitic character of some of the other organs in connection with the etiology.

CASE OF UNILATERAL THROMBOSIS OF CEREBRAL VEINS.

GEORGE P. BIGGS, M.D.

The following condensed record of this case is of interest, partly because of inability to harmonize the symptoms with the lesions. The patient was a male, aged twenty-seven, a native of Ireland, single, mattress maker. He gave a history of syphilis four years ago. Moderate drinker. Two weeks ago noticed weakness and anorexia. Pain in right axilla; general aching pains. After five days was able to be out. One week before admission had a severe chill followed by fever and pain on right side. Cough with mucopurulent expectoration, later becoming blood-tinged. Signs of consolidation in the lower part of the right upper lobe and apparently in the upper part of the right lower lobe. On the third day after admission he looked septic and it was suspected that there might be some pus in the right pleural cavity. A needle was inserted with negative result. Immediately following this aspiration, the patient became pale, pulse imperceptible, respiration labored. After stimulation, considerably improved. On the same day there is an entry on the chart of slight nystagmus of the left eye. At five-thirty

of the same day, the nurse reported a right-sided convulsion lasting about two minutes. On the following day (the fourth day after entrance to the hospital), another slight right-sided convulsion, lasting about half a minute.

On the fourth day of the man's stay in the hospital, it was recorded that since the previous afternoon the patient had been unable to move the right arm or the right leg, or left side of the face. The right arm and leg were rigid. Knee-jerk seemed more active on the right than on the left side. Patient semi-comatose. On the morning of the fourth day, it was impossible to make the patient move the left arm or the left leg; the right side of the face was movable.

On the fifth day, the left side of the body was only slightly rigid, and the left hand responded to stimulation.

On the sixth day, patient moved both arms on irritation. Pulse weak, respiration labored. Died on the seventh day after admission, two weeks after definite chill with pneumonic symptoms.

Temperature, 104° on admission, inclined to become lower, reaching as low as 100° the day before he died; rising the night before death to 104°. Respiration varied from twenty-four to thirty-six. Leucocytes, 13,800. Embolism was thought the probable explanation of the cerebral symptoms.

At autopsy numerous old adhesions were found over the liver, also pleuritic adhesions on both sides. Heart normal, except that the aortic cusps presented a slightly roughened appearance suggesting acute endocarditis, although the lesion was not marked, also a nodular area of thickening of one of the cusps, three mm. in diameter.

After the pulmonary vessels of the right lung had been opened very freely with scissors, three small portions of ante-mortem thrombi were found. Their origin could

not be determined but they must have been loosely attached in some branch of the pulmonary artery or vein. No other thrombi were found in the lungs. There was considerable broncho-pneumonia of the right lung, particularly in the region in which the physical signs had been heard, an also fairly extensive chronic interstitial pneumonitis of the right lung. A small area of obsolete tuberculosis was found in the right apex. In the kidneys there was parenchymatous degeneration with a few hyaline casts in the tubules of the pyramids.

The most noticeable lesions after the removal of calvarium and dura were areas of diffuse hemorrhage of the pia over the right hemisphere, adjacent to and extending a distance of about four cm. from the great longitudinal fissure. There was no clots of blood. Veins over the right hemisphere were distended and in contrast with those over the left hemisphere. Convolutions on both sides were moderately flattened.

Having in mind the possibility of emboli or thrombi at the base of the brain, very thorough search was made, the vessels being carefully watched as they were severed and after removal of the brain by careful dissection. There was absolutely no trace of thrombi or emboli. The right Sylvian artery did not appear to be especially engorged and there seemed to be nothing especially noticeable about the arterial supply as far as the large vessels on the right side of the brain were concerned. On opening the longitudinal fissure a parietal thrombus was seen loosely attached to the right wall. It was three cm. in length and located midway between the anterior and posterior ends of the sinus. The terminal ends of the right cerebral veins emptying into the sinus in the region of thrombosis contained occluding thrombi, one cm. in length. Beyond the thrombi the veins were distended

with fluid blood. These thrombi corresponded with areas of hemorrhage noticed in the pia. Parietal thrombi were found in each lateral sinus, also. Apparently nearly all of the right hemisphere above the level of the roof of the lateral ventricle was softened, but much of this must have been the result of pronounced oedema as the appearance of the hardened specimen does not bear out the original impression. Softening is limited to the regions of hemorrhage, most of which correspond with hemorrhages in the pia. These areas of cerebral hemorrhage associated with evidences of softening have a curious outline, often following the irregularities of the cerebral surface as a cortical zone three to seven mm. in thickness. Depth of involvement thus depends partly upon the depth of the sulci. These areas of hemorrhage are entirely in the right hemisphere, chiefly near the great longitudinal fissure. They are one to three cm. in diameter, and the intervening tissue is fairly normal. No evidences of such lesions are seen in the left hemisphere.

The microscopic appearances of the cerebral tissue are very confusing. In the first place there are evidences of a probable syphilitic lesion in the form of hyaline degeneration of some of the vessels. Then there are other vessels in the regions of softening and hemorrhage, which are occluded with thrombi, some of them fibrinous, some rich in leucocytes. In some instances the perivascular spaces are filled and the surrounding tissue infiltrated with small round cells; in other instances there is no infiltration of surrounding tissue. The inflammatory infiltration is a marked feature in many parts of the involved tissue. Hemorrhages are very numerous, at times very closely related to the thrombosed vessels and at other times hemorrhages are found about vessels which show no distinct lesion. In places, chiefly in the sulci,

the pia shows slight round cell infiltration and endothelial proliferation. The question naturally had arisen as to the correct explanation of the lesion. Grossly, the only demonstrable interference with circulation was in the cerebral veins where it was very distinct as shown by the engorgement of the veins beyond thrombi. The arteries, so far as it was possible to trace them by dissection, did not contain thrombi or emboli, and none have been found in the sections. Dr. Biggs wished very much to have some expression of opinion as to the sequence of events and nature of the lesion.

LARGE ANEURYSM OF THE AORTA.

FRANCIS C. WOOD, M.D.

Dr. Wood presented a specimen of aneurysm of the aorta. He said that as a rule pathological specimens were not interesting on account of their size; but he had had an opportunity of removing a large aneurysm from a patient which had excited some interest, and he thought the members of the Society might like to see the extent to which such a growth might develop.

It had been removed from a large and powerfully built man, about forty years of age, who had entered St. Luke's Hospital with an aneurysm which had already eroded one of the ribs. He had symptoms of aneurysm and the history of syphilis which so often goes with it. He was supposed to be in a dangerous condition, so much so that upon asking for an X-ray picture it was said that the man was moribund and could not have the picture taken. He survived this moribund condition, however, for four months, and was a comfortable, cheery mem-

ber of the Hospital wards. As the aneurysm grew larger, it did not seem to produce much discomfort. At the time of autopsy, it projected forward four inches from the anterior surface of the right chest. It seemed as if it would burst at any moment. It had eroded the border of the sternum and extended to the axilla. The ribs were eroded from the first to the seventh and were included in the mass, The lung was laterally compressed, and the pleural cavity contained a small amount of blood where the aneurysmal sac had leaked. The were also small hemorrhages under the muscles of the chest. The heart had remained comparatively small for so large and powerful a man. The opening of the aorta into the aneurysm was closed by a clot. The patient had practically undergone a spontaneous cure, as very little blood could be obtained from the aneurysmal sac. In a few weeks he might have recovered.

PRIMARY EPITHELIOMA OF THE BRONCHUS.

O. H. SCHULTZE, M.D.

Dr. Schultze presented specimens from a case that was diagnosed clinically by Dr. I. Adler as Primary Endothelioma of the Lung. He was indebted to Dr. Maurice Packard who treated the case for clinical history. Male, 54 years, single, cigarmaker, an inveterate smoker. Family history of arterio-sclerosis, no evidence of tuberculosis, syphilis or alcoholism.

Two years ago he had a cough which although alleviated by treatment never entirely left him. There were

no night sweats, no loss of flesh or strength, no hæmoptysis at this time, no dyspnœa. The cough during the last two years gradually became worse and about 18 months ago was accompanied with profuse mucous expectoration. Dyspnœa became very troublesome and he now suffered from pain localized in anterior right side of chest and increased on deep breathing. Sputum was examined for tubercle bacilli with negative result. He was sent to the South but as he did not improve he returned. The cough became more distressing and on many occasions a considerale amount of blood, as much as a cupful, was brought up. Even after these hemorrhages he did not lose weight, nor was his temperature higher than normal.

There was flatness on percussion over upper and anterior part of right side of the chest and the veins over this region were prominent.

Death occurred suddenly with profuse hemoptysis.

Autopsy. The right pleural sac contained about one quart of blood stained serum. The anterior surface of the right upper lobe was intimately adherent to the costal pleura which was removed with it. Larynx, trachea, bronchi, lungs, heart and vessels were removed together. A firm tumor 3 c.m. by 5 c.m. involves the posterior wall of the right bronchus, the bifurcation of the trachea, and beginning of the left bronchus. The bronchi especially the right are partially stenosed and ulcerated. Immediately below this mass a bronchial lymph-node about 1.5 c.m. by 3 c.m. is found almost completely infiltrated with new growth. The growth extends along the bronchi into the right upper lobe to its anterior and internal surfaces where the pleura is intimately adherent to the pericardial sac. Dense infiltration of the growth surrounds the pulmonary vein and artery and the superior

vena cava. The lumen of the superior vena cava and that of the pulmonary vein present mere dimples with radiating longitudinal folds of the vessel wall when viewed from the auricles. The lumen of the right pulmonary artery is narrowed to 5 m.m. Sections of the growth throughout the lung are firm with the exception of areas of erosion into the bronchi evidently the source of the hemorrhage.

The middle lobe presents firm consolidation of yellowish-white color and flat on section. There are scattered small metastases in the pleura and about the bronchi of the right lower lobe which was compressed by the fluid in the pleura, in the pleural surface of the diaphragm and throughout the left lung. A few isolated metastases up to 5 m.m. in diameter were found in a "horse-shoe" kidney.

The microscopical sections presented show the same structure throughout, variously shaped islands and strands of cells without intercellular substance, imbedded in a stroma of dense fibrous connective tissue. In these islands the outer cells are polygonal with large vesicular deeply staining nucleus and basophilic protoplasm. Toward the centre the cells become flatter, stain more distinctly with eosin and their nuclei stain lighter with hæmotoxylin. Concentric arrangement of these flattened cells is distinct in many places. With Weigert's stain granules and plates of keratohyalin are found here. In many islands and in the small metastases, the outer cells present evidences of growth and infiltration of the surrounding tissue while the inner cells show progressive loss of struture, coagulation necrosis. The ulcerated surface of the tumor in the wall of the bronchus presents a hemorrhagic granulation tissue. Smooth muscle fibers of the wall of the bronchus can be traced through the tumor.

ADRENAL TUMOR WITH METASTASES.

O. H. SCHULTZE, M.D.

The patient from whom these specimens were obtained, a man 52 years of age, died in Gouverneur Hospital 40 minutes after admission. He was suffering from intense dyspnœa, no pulse could be felt and he presented signs of edema of the lungs. The immediate cause of death was excessive edema of the lungs, dilatation and fatty degeneration of the heart, due to secondary pernicious ænemia in turn dependent upon malignant new growths.

The left adrenal presents a tumor occupying nearly the entire organ which is symmetrically enlarged to four times its normal volume. The right kidney is almost entirely replaced by a large growth in volume nearly eight times that of the normal organ, only a small portion of the organ being recognizable. The growth extends through the renal vein into the inferior vena cava distending it to twice its normal diameter, as far as the liver. The pelvis, ureter and bladder contain blood clot. The lower epiphysis and adjoining portion of the shaft of the right femur is increased in size to four times the volume of the left. Absorption of bone had advanced to such a degree that the mass was easily broken from the rest of shaft, and sections through the mass were readily made with the knife. Numerous isolated metastases were found in pulmonary pleura and about the bronchi also in the left kidney.

Microscopical slides were presented from all the growths. The structure presents a reticulum of thin walled capillaries, the spaces occupied by large polygonal cells whose nuclei stain intensely, the protoplasm, however, very faintly. In the adrenal, kidney and femur numerous giant cells are found with central, close arrange

ment of the nuclei, probably produced by agglutination of cells. In the metastases in the vena cava the cells are compressed and fusiform and the vascular reticulum less distinct. The structure corresponds to that of the adrenal cortex. The tumors have been described in the adrenal as Stroma adrenalis or suprarenalis in the kidney and elsewhere as Hypernephroma, adrenaloma or Perithelioma. The kidney tumor may have originated from an embryonal adrenal nest.

INTRA-PERITONEAL HEMORRHAGE FROM ADENO-CARCINOMA OF THE LIVER SECONDARY TO ADENO-CARCINOMA OF THE GALL-BLADDER.

O. H. SCHULTZE, M.D.

The patient, a laborer 43 years of age, died suddenly twelve hours after admission to Bellevue Hospital. Dr. Samuel A. Brown has kindly furnished the following clinical notes. The patient had lost 38lbs. during the last three months. One month before admission noticed that his eyes were yellow, the condition was progressive with emaciation, weakness and loss of appetite. Five days before admission he began to have acute pain on the right side over the liver, increased on pressure and on taking a deep breath.

Temparature on admission 101°, signs of cardiac insufficiency and acute peri-hepatitis. While on bed-pan endeavoring to defecate he toppled over and died within an hour.

Autopsy. Two quarts of blood found in the peritoneal cavity. The source of the hemorrhage is found in a laceration of the capsule of the liver communicating

with a large hemorrhagic and softened metastasis The liver is considerably increased in size and filled with numerous large metastases hemorrhagic and softened. The superior surface of the gall-bladder is intimately adherent to the liver and presents a papillary growth on its mucosa, infiltrating its walls and the liver. The retroperitoneal lymphnodes about the head of the pancreas, pylorus, and hilus of liver, are considerably increased in size, infiltrated by new growth, hemorrhagic and softened. The retroperitoneal connective tissue is infiltrated with blood, pushing the pancreas forward. The entire mass is about the size of a man's hand. The pancreas, however, is normal.

Microscopical slides of the primary tumor and metastases were presented.

The structure is that of adeno-carcinoma with high, columnar cells, in places arranged in tubules, in others irregularly infiltrating the surrounding tissue. Extensive necrosis and hemorrhage is apparent.

Discussion.

Dr. Noyes said that three or four years ago he had presented to the Society a large tumor of the liver of adenomatous nature which when put under the microscope proved to be nearly identical with suprarenal gland tissue. It had caused some discussion and a marked difference of opinion, some of the members believing it to be simple ordinary adenoma, others believing it to be metastatic deposits of the ordinary cells of the suprarenal gland in other organs; but the speaker believed it to be identical with the specimen shown by Dr.

Schultze. It was one very large tumor mass which, seen in the specimen alone, would have been taken for suprarenal capsule if it had not been known where it came from. No primary growth was found in the suprarenal gland.

Dr. Harlow Brooks said it was most interesting to study these cases and to try to find out what primary tumors of the lung were and where they started. He had studied three cases of so-called primary endothelioma of the lung, two of them so named with his half-hearted consent, and one with his vigorous opposition; he was absolutely sure this one was carcinoma. He thought these cases were too frequently called endothelioma because they were primary tumors of the lung; and perhaps this was because the best work in this line had been done by a man who believed in endothelioma with all his heart. He had been glad to hear Dr. Schultze's explanation of this tumor. Dr. Brooks said it was necessary to consider in primary tumor of the lung the embryological epithelium from which the lung is developed—air sacs—derived from a type of epithelium — endoderm — which does many curious things, as in the intestine, liver and other organs similarly derived. When we come to the new growths, such as endothelioma we must remember we are dealing with tumor cells which from an embryological standpoint are apt to produce very variable tissues even in the normal. Dr. Brooks did not think the average primary tumor of the lung was an endothelioma, but he was not prepared to say all were not endothelioma.

Dr. Biggs asked as to the experience of others in regard to the malignancy of hypernephromata. Personally he had never seen metastasis or recurrence and he

was inclined to give a favorable prognosis if the tumor were sharply outlined and not large.

Dr. Schultze said that in a case at Mt. Sinai Hospital of a pulsating tumor of the ilium diagnosed clinically as aneurysm, exploratory incision revealed a mass containing blood. The microscopical diagnosis made by Dr. Mandlebaum was perithelioma. Such metastases of hypernephroma in bone were not so frequent. Stroma suprarenalis (hyperplasia of adrenal cortex) he considered not so uncommon if the adrenals were systematically examined. Hypernephroma of the kidney was occasionally found, apparently having no connection with the cause of death.

Dr. Brooks said that he had had two cases last year; one in the Montefiore Home and one in the Fourth Division of Bellevue. The first was diagnosed antemortem and showed the extremely rare metastases in the corpora cavernosa. He did not think them so uncommon if one looked out for them.

Dr. F. C. Wood said that he had seen a tumor of this type removed from a patient at St. Luke's Hospital. The tumor had occupied the upper lobe of the kidney and was of very considerable dimensions and filled with fluid blood. The inner surface of the tumor was covered with a shaggy mass of new growth. Evidently there had been a hemorrhage in the substance of the tumor with consequent destruction of the cells and the formation of the cyst. The patient had gone five years without a recurrence.

Dr. Schultze said that it seemed strange that the case with the adrenal metastasis in the inferior vena cava had not shown more marked effects of interference with circulation.

POLYCYTHEMIA IN CONGENITAL HEART DISEASE.

(*Preliminary Notice.*)

IRA S. WILE, M.D.

The polycythemia of the first few days of infancy is well known. Schiff accounts for it on the basis of increased metabolic activity demanding an increase of red cells to provide oxygen for the cells. The infant at birth has an increase of red corpuscles as compared with the mother. Delay in ligation of the cord increases the infantile polycythemia. The processes of respiration and nutrition are carried on for the fetus in the placenta. A large number of red corpuscles is necessary to provide proper oxygenation of the fetal cells. In congenital heart disease there is a persistence of fetal conditions, though a larger volume of blood goes to the lungs for oxygenation. According to the *American Textbook of Physiology*, hemorrhage causes loss of red corpuscles and consequent loss of oxygen for the tissues. This brings about starvation of the hematopoietic tissues as a result of which the erythroblasts are stimulated to the formation of new cells. In congenital heart disease a limited porportion of corpuscles succeeds in getting from the right auricle through the right ventricle to the lungs. As a result a lessened amount of oxygen could be supplied to the cells if the number of red corpuscles remained unchanged. To supply this oxygen deficiency, I believe a compensatory production of red cells takes place. Each cubic millimeter then contains an increased number of cells capable of carrying the oxygen needed for cell nutrition and activity. Polycythemia of congenital heart disease is regarded as largely dependent on cyanosis.

Mixture of arterial and venous blood, blood stasis from pulmonary obstruction, blood concentration, are the main causes enumerated. Most writers accept the theory of Grawitz that blood concentration is the cause. If inspissation is the cause of the polycythemia we should expect to find an increase of leucocytes somewhat parallel with that of the erythrocytes. From a chart of white and red cell counts it is noted that the average white cell count was 10,256; while the red count was 7,945,615. The ratio is 1 to 775—below the usual ration. As most of the counts were on children where white counts are usually large, the white cell average may be regarded as perfectly normal while the red cells are greatly increased. From fifteen reported red cell counts and hemoglobin estimations, I find that while the average increase of red corpuscles above 5,000,000, the unit of comparison, is .5022, the average hemoglobin is 107.5, and the average color index, 73.5%. The color index is 26.5% below normal, while the red cells are increased 50.22%. On the inspissation theory this increase of erythrocytes would require loss, drain, or evaporation of serum to two-thirds of its bulk—a manifest absurdity. If blood be regarded as a tissue with fluid intercellular substance, it is reasonable to believe in hypertrophy of its various constituents. To compensate for the lowered hemoglobin content per corpuscle and the lessened frequency of pulmonary oxygenation of each corpuscle, it is necessary to increase the number of oxygen carriers in each cubic millimeter of blood.

Therefore, I believe that the logical explanation of polycythemia of congenital heart disease lies in an actual polycythemia because of suboxidation. This will explain polycythemia where cyanosis does not exist and where cell nutrition appears unimpaired.

QUANTITATIVE ALBUMIN DETERMINATIONS.

KARL M. VOGEL, M.D.

In spite of the fact that the importance of the subject has stimulated the investigators of several generations to endeavor to produce some easily applicable, rapid method for the quantitative determination of the albumin in the urine, their efforts have thus far been fruitless. The number of reliable qualitative tests which can be quickly carried out is quite large, but the only quantitative procedure which is entitled to consideration where accuracy is required, is the tedious and complicated method of drying and weighing the precipitate obtained on coagulating the albumin by heat.

Of the methods applicable to routine clinical work, those of Esbach and Purdy have perhaps the greatest claim to convenience and ease of execution. The errors attending the use of the Esbach tube are too well known to require comment, and in addition a delay of twenty-four hours is necessitated before the result can be obtained. The centrifugalization method of Purdy is also open to criticism on the score of variations in the density of the deposit due to differences in the specific gravities of the urines tested, and owing to the technical difficulties of ensuring an absolutely uniform speed of the centrifuge at exactly the prescribed rate of 1500 revolutions per minute during the required length of time.

The convenience of volumetric methods has naturally led to efforts to introduce them into this field also. Tanret (1), Boedecker (2) and Venturoli (3), years ago devised analytical schemes of this sort, but they have been discarded as untrustworthy. Sahli (4) has lately proposed a volumetric method in which the albumin is

precipitated by heat, filtered off, and re-dissolved in a known amount of alkali and its amount determined by titrating with an acid, using congo red as indicator; but he himself expresses doubts as to the reliability of the figures obtained in all cases.

The simplest and most promising of the volumetric procedures is that published by Wassiliew in the *St. Petersburg med. Wochenschrift, No. 37, 1896.* The author's description of the method has been reproduced, but without comments as to its reliability, in various publications, and Ditman about a year ago called attention to the possible availability of the method. The present study was undertaken as a continuation of his determination in order to establish the accuracy of the results to be obtained by this plan.

The method consists in the precipitation of the albumin by a 25 per cent. solution of sulphosalicylic acid, using an aniline dye known as *echtgelb* as an indicator and then multiplying by an empirical factor to obtain the amount of albumin. It was found possible to simplify somewhat the technique of the process without in any way invalidating its principles. The sulphosalicylic acid solution, which of course should be as accurately standardized as possible, can quickly be prepared by making it of such strength that it exactly neutralizes two c.c. of a N/1 KOH solution, using phenolphthalein as an inditor. When made in this way, Wassiliew's factor, which consists of five decimals, may be reduced to the fraction .01 and if ten c.c of urine be taken for the test, the percentage of albumin is obtained directly by noting the number of c.c. of sulphosalicylic acid required to produce the end reaction, and then moving the decimal point one place to the left.

The end reaction is by no means an easy one to de-

termine, especially if the quantity of the indicator, two gtts., recommended by Wassiliew, is employed. It was found that the use of ten to twelve gtts. produced a much sharper end reaction without any apparent decrease of sensitiveness, the reaction being, if anything, more delicate with the larger amount.

The most advantageous technique was found to be as follows: Several portions of ten c.c. each of the urine measured with a pipette were placed in flat evaporating dishes of about 7 cm. diameter. If necessary the urine was acidified with acetic acid, a frankly acid fluid appearing to give the sharpest end reaction. The same number of drops of indicator was added to each portion and the reagent then very slowly run in from a burette with constant stirring, as the union of the acid and albumin appears to take place somewhat slowly. The first portion tested was taken as a control, and after the end reaction had been reached as denoted by the production of a red color which did not appear to be deepened by the addition of a further drop of the reagent, a slight excess was run in. The titration was then carried out on the other portions in dishes of the same nature and under the same conditions of acidity and quantity of indicator as the first, the end reaction being reached as soon as the same shade of color as the control was obtained. Carried out under these precautions titrations on several portions correspond very closely.

In order to control the results obtained a number of gravimetric determinations of each urine were made and accurate averages thus secured. Six specimens of urine were used and each was also tested volumetrically, after dilution to one-half and to one-quarter with normal urine, thus making eighteen different albumin contents. It was found that the volumetric method gave fairly accurate

results for urines containing more than .15 to .2 per cent. of albumin (the highest strentgh of the urines tested was .27 per cent.) but that below this limit the percentage of error was so inconstant and high as to deprive the method of all claims to accuracy, The following table showing some of the averages obtained will serve to indicate the general character of the results yielded by the method.

No. of Specimen	Amount of Albumin in 100 c.c. Gravimetric Method	Concentration of Urine	Amount of Albumin in 100 c.c. Titration Method
I	.245	Undiluted	.245
		Diluted to ½	.164
		Diluted to ¼	.140
II	.137	Undiluted	.133
		Diluted to ½	.121
		Diluted to ¼	.120
III	.222	Undiluted	.224
		Diluted to ½	.130
		Diluted to ¼	.122

An important source of error appears to be the fact that even in normal urines a certain amount of sulphosalicylic acid must be added before the end reaction is obtained and as this is not a constant quality it would seem to be an irremediable defect of the method. It may be remarked in passing that the control determinations on which Wassiliew based the procedure were made by precipitating the albumin of the urine with alcohol, a method that has been found very unreliable.

Since the above study was begun, two authors, Spaeth (5) and Gerard (6), have in their volumes on urinalysis just published mentioned the method. Spaeth condemns it utterly and Gerard ranks it with the roughly approximate methods.

Bibliography.

1. *Bull. de therap.* 7, 1877. *Centralblatt für die med. Wissenschaft.* 1877. 493.
2. *Ann. d. Pharm.* 111, 195. L. Thomas, Schmidt's Jahr. 120, 171.
3. *Chem. Centralblatt.* 1890. 2, 525.
4. *Lehrbuch der klinischen Untersuchungsmethoden.* 1902.
5. *Die chemische und mikroskopische Untersuchung des Harnes.* 1903.
6. *Traité des Urines.* 1903.

Discussion.

Dr. E. E. Smith said that Dr. Vogel had referred to the fact that in the application of the method, a variable amount of the reagent added was used to react with other constituents than albumen. This was a variable factor and interfered with the accuracy of the test to a certain extent. Dr. Smith inquired whether Dr. Vogel had considered the advisability of running a control by separating by the heat method the albumen from a portion of the urine, filtering it off and running a control on the filtrate as a factor for correction, That would take but a few moments—acidify the urine with acetic acid, heat and filter and run a control on the filtrate. It seemed to him that would afford a correction.

Dr. Vogel said he understood what Dr. Smith meant and it might possibly afford a factor for a particular urine.

Dr. Smith said that he wanted to call attention to the fact that Esbach's method so generally used in office examinations was quite unreliable owing to the fact that the tubes manufactured are made on a wrong principle at the present time. The original principle upon which the Esbach tubes were made was the fact that the albumen precipitates out by gravity a certain distance in a certain

time. The tubes should have the "U" mark at a certain uniform height. The manufacturers had made the mistake of supposing that the "U" mark should represent the capacity of the tube. Consequently instead of being at a fixed height the "U" marks in the tubes now used varied greatly in position, with the result that there was a variation of 50%, more or less, in the results obtained in the different tubes. Dr. Smith thought the fact important and that perhaps those who had not considered it would like to have their attention called to it.

Dr. P. A. Levene said that for clinical purposes the Esbach method was quite satisfactory. The presence of a few milligrams more or less of proteid in the urine does not indicate any alteration in the conditions of the kidneys. It was of importance to have a general knowledge whether the urine contained small or large quantities of albumin and a similar knowledge of the decrease or increase of the substance in disease. Such knowledge it was possible to obtain by Esbach's method. Dr. Levene did not believe that it would be possible to devise a reliable volumetric method for the estimation of proteids, for the reason that proteids combine with other substances very slowly.

Dr. Geo. Mannheimer said that he had not often made quantitative estimates of albumin in urine, because they seemed to him not of great clinical value. The amount of albumin he considered diagnostic only in certain conditions of the kidney; e. g., congestion due to heart disease, where it varied with the state of the circulation. In cases of chronic nephritis, the degree of albuminuria varied so much from day to day that it could not serve as a guide to the severity of the disease. Von Noorden brought out, years ago, the fact that the variations were so great as to be almost characteristic of

chronic Bright's disease. In a few cases where it had seemed of interest to estimate how much albumin was passed in twenty-four hours, he had determined the total nitrogen by the Kjeldahl method, first with the albuminous urine, and then with a thoroughly desalbuminized specimen of the same twenty-four hours collection. The difference between the two results indicated the amount of albumin. This procedure had been compared with the weighing method as a standard and the error had been found to be less than $\frac{1}{100}$ of one per cent. Esbach's method in spite of its faults seemed a convenient clinical one to use for continuous estimation in a given case. If employed always under the same circumstances, the same mistake would always be made and it could thus be used for comparison. Dr. Mannheimer asked Dr. Vogel whether he had tested the method he described against any other reliable one.

Dr. Smith said he had not anticipated Dr. Levene's remarks on the Esbach method. In view of the fact that Dr. Levene inferred that the Esbach method was used by all, he thought it would be well to describe the method he used in his routine work. It was quite simple and had an advantage over the Esbach method in that it took less time to get final results. It was practically a simplified gravimetric method. He took a small quantity of urine, an amount that he estimated would contain about twenty milligrams of albumin. This he diluted with a saturated solution of common salt, coagulated with heat and acetic acid; let it stand on a water bath for a few moments until the coagulum was well formed, and filtered through a Gooch crucible, or through a small, weighed, folded filter. The precipitate was washed with hot water, alcohol, and ether. All this took but little time and for anyone who did it in a routine way it was very simple.

The Gooch or filter was dried in an oven for three hours at 110°—a much more than ample time to thoroughly dry the precipitate. It is then cooled and weighed, and in the case of the Gooch, ignited and re-weighed. This gave a ready method for the estimation of albumin; in the laboratory it could be done in half a day; and the method was much more satisfactory than that of Esbach, and one that the speaker thought would be followed with advantage by those who had the equipment of a laboratory.

Dr. Vogel said that the control determinations were made by the method of Scherer which consists in carefully acidulating with acetic acid and then coagulating the albumin by heating on the water bath for one-half hour. The speaker added a certain amount of salt solution before heating in order to ensure complete precipitation of the albumin. The fluid was then filtered through a dried, weighed filter; the precipitate washed with water, alcohol and ether, dried to constant weight, and the amount of albumin determined. That, he thought, was the method usually considered the most accurate. Incineration of the filter was not necessary if the amount of precipitate were not large and the washing was thoroughly done. The study had seemed advisable for the reason that this method of Wassiliew's has been reproduced in a number of different textbooks, always without any special comments, which indicated that it had been accepted. Dr. Vogel had thought it did not seem right to have the method go on, perpetuated indefinitely, without investigation, so he undertook it. The result had been rather unsatisfactory to the method.

As to Dr. Levene's remark that no tritation method of determining the amount of albumin was likely to be successful, Dr. Vogel said in this investigation everything

had pointed to that fact. It appeared that the union of the sulpho-salicylic acid with the proteïd took place quite slowly and evidently incompletely, so that there was no definite means of knowing when complete union had taken place. Probably, also, the fact that albumin which originates from different sources may have different affinities for acids, entered into the question.

As to Dr. Smith's remarks, Dr. Vogel said that in carrying out the determinations a large number of parallel determinations with the Esbach tube had been made. He could only corroborate what Dr. Smith had said as to the unreliability of the Esbach method. The gravimetric method described was extremely accurate but, for the practioner, entirely impracticable. He thought a method which could be applied to the purposes of daily routine use without such expenditure of time, and yet would afford fairly reasonable accuracy, was the desideratum. Some German authors used tables from which the amount of weighed albumin corresponding to given bulks of precipitate settling in the test tube after boiling, and roughly gauged by the eye, might be determined. This method was open to gross errors, but was said not to be more so than the Esbach. It was not necessary for clinical use to know the exact number of milligrams to the liter but to know whether the amount was increasing or diminishing from day to day.

SOME OBSERVATIONS ON COMPLEMENT.

EDNA STEINHARDT, M.D.

Dr. Steinhardt read some observations on complement. The work had been carried out in the Research Laboratory of the Health Department, through the kind

permission of Dr. W. H. Park, whom she wished to thank for his courtesy and for his many suggestions and criticisms. Her thanks were also due to Dr. Anna W. Williams for her suggestions during the course of the work.

The points under consideration were:—

1.—The effect on the complement of passing serum through a Berkfeld filter.

2.—The differentiation of bacteriolytic complements by means of a filter, by heat, and by preservation.

It was found that the fresh serum of the horse was lytic to the stock typhoid culture, so this combination was used in the following experiments.

When fresh lytic serum was passed through a small Berkfeld filter, the first ten c.c. had lost its lytic action, the second ten was partially active, the third more so, while the fourth ten was unimpaired in its lytic strength. This gradual passage of the lytic property of the serum through a Berkfeld filter accords with the same fact noted by Graham-Smith, for the precipitins (1), and by Park and Collins for the agglutinins (2). There are, however, in lytic action, two factors to be considered, the immune body and the complement. The question is: Is the first filtrate lacking in one or both. Ehrlich and Morgenroth and Ainley Walker state that the immune body is present in the filtrate; while Pfeiffer, who filtered small quantities of serum, noted considerable reduction of the immune body.

To test this point, portions of the first ten c.c. passed through the filter were added to fresh lytic serum and the Neisser and Wechsberg phenomenon of deviation of the complement, due to excess of immune body, was pro-

(1) *Journal of Hygiene.* July, 1903.

(2) *Proceedings of the New York Pathological Society.* Jan., 1904.

duced. Therefore, though there may have been a diminution of immune body in the filtrate, still there was sufficient present to produce lysis, if there had not been a lack of complement. Ehrlich and Morgenroth state this retention of complement to be due to adsorption to the filter. This was proven to be so by passing ten c.c. of a lytic serum through a small Berkfeld filter. Five c.c. of this filtrate were then drawn through the filter in the reverse direction. The first filtrate was tested and found to be non-lytic; while that portion which had been passed back through the filter was lytic to a certain extent, though not to the same degree as the original serum. This difference in strength is easily explained by the fact that it would be impossible with such a small amount of fluid to wash the filter clear.

This same fact of absorption was shown by another method: namely, ten c.c. of the lytic serum were drawn through a Berkfeld filter. The filtrate was removed and proven to be inactive. Then twenty-five c.c. of .8 per cent. NaCl solution were drawn through this filter in the same direction. This washing was evaporated to dryness in a vacuum over sulfuric acid at room temperature, and the residue when dissolved was distinctly lytic. To control this, lytic unfiltered serum evaporated in the same manner was shown to still retain its lytic properties, while neither inactive evaporated serum, nor concentrated NaCl solution had any lytic effect when tested. It was also shown that if twenty-five to thirty c.c. of inactive serum were passed through a filter and then ten c.c. of a lytic serum were drawn through, this last serum would still retain its lytic quality. In other words, the coating of the filter is purely mechanical, the filter having no selective action for complement. The complement, however, usually exists in serum in so much smal-

ler proportions than the immune body that in the first few c.c. of a filtrate it is probably present, though in such minute quantities that it is impossible to detect it with our present technique.

Ehrlich and Morgenroth found that they were able to differentiate two hemolytic complements by filtering serum. This was corroborated by Neisser and Döring. Later Vedder repeated this for the bacteriolytic complements. There are three hypotheses that suggest themselves as possible explanations for this separation of complements of a filter.

1.—The difference in size of the molecules of the different complements.

2.—The difference in the adhesiveness of the molecules.

3.—The difference quantitatively of the various complements.

As to the first, it seems rather improbable that a molecule should be large enough to block the pores of a Berkfeld filter, which are comparatively coarse. The second may be the explanation; but the next hypothesis seems the more probable one, as indicated by the results so far obtained.

In rabbits' serum the greatest lytic action occurs for the typhoid bacillus, less for the dysentery and least for the colon; and when Vedder filtered rabbit's serum, he found absence of lysis for the colon, impaired for the dysentery, and unimpaired for the typhoid organisms. To take the agglutinins as an example, in a serum which contains twice the amount of agglutinins for Flexner "Baltimore" as it does for Penman (two dysentery organisms), when a few c.c. of this serum were filtered and this filtrate tested, it was found that the agglutinins were reduced proportionally; that is, two-thirds of each had

been retained in the filter. It would be possible by the testing of successive filtrates to find that point where no visible reaction could be obtained with Penman, but Flexner "Baltimore" would still in certain dilutions be agglutinated; thus apparently differentiating by a filter between the two agglutinins. The tests for small amounts of agglutinins are, however, much more delicate than those for complement. For this reason, with the present methods a trace of complement could not be detected and would be called absent. Therefore, it seems probable that the differentiation by the filter of bacteriolytic complements is a quantitative rather than a qualitative one.

Tests were also made upon the differentiation of bacteriolytic complement by heat, and here the typhoid complement was found to be more resistant than the colon or dysentery complement, but the serum originally was more lytic towards typhoid than towards either of the other two. Also, the complement for the typhoid bacillus was present longer in preserved serum than was that for the dysentery organism.

It may be that the differentiation of bacteriolytic complements by heating, and by permanency, as well as by filtration, is explainable by the same theory of quantitative differences in their amounts originally present in the serum. However, as yet this is only an hypothesis suggested by the similarity of results obtained in all three experiments, and further work will have to be done on these points, before this theory can be proven or disproven.

In conclusion, the results obtained were summed up as follows:

1.—The gradual passage of bacteriolytic complement through the Berkfeld filter.

2.—The retention of complement of the first portions of the filtrate by the filter being due to absorption.

3.—The probable explanation of the differentiation of bacteriolytic complements by filtration is their quantitative differences in the original serum.

4.—The similarity of results obtained by the effects of heat and by the effect of time on certain bacteriolytic complements, suggests their explanation on the same basis.

Discussion.

Dr. P. A. Levene remarked that the observations of Ehrlich that certain complements did not pass Berkfeld filters might be explained by the supposition that they were in colloidal solution. The more soluble complements would pass through the filter more readily. It was not of so much importance to know whether the Berkfeld filter is absolutely or partly impermeable for a complement, as it is to be able to separate the substances by means of filtration. Ehrlich might have achieved this owing to his great experience in handling these substances, and to his knowledge of their behavior under different conditions.

Dr. Steinhardt replied that there was no doubt at all of the separation of complements by means of a filter, as shown by Ehrlich, and repeated by other workers. The question had been only of the reason for this separation. The supposition of the colloidal nature of some of the complements causing their retention in the filter might be correct. It was rather difficult, however, on that basis, to understand the passage in the first few cubic centimeters of serum, of the complement for the typhoid bacillus through a filter previously coated with

inactive serum, which complement would be completely retained by using an uncoated filter. Also the proportionate reduction in filtered agglutinins and the similar results obtained for bacteriolytic complements suggested the probable explanation as being a difference in the several complements of quantity rather than quality.

ON THE PRESENCE OF MALLORY'S CYCLASTER SCARLATINALIS IN THE SKIN OF SCARLET FEVER PATIENTS.

CYRUS W. FIELD, M.D.

Dr. Field spoke on Mallory's Cyclaster Scarlatinalis in the skin of scarlet fever patients. He said that these bodies were described by Dr. Mallory before the Society of Medical Research in Boston, on the fifteenth of December, his paper being published in the January number of the *Journal of Medical Research*, *1904*. He had found the bodies first in the skin of a boy who had died on the third day of the rash. There were three specimens examined, one each from the thorax, abdomen and thigh. One of these pieces showed the bodies present in the epithelial cells, between the lymph cells, and in the lymph space of the corium. In another piece there were a few and in a third none at all. Dr. Mallory had examined this specimen as he wished to compare forms of degeneration in scarlatina with those found in variola and had found bodies he had noticed before. He had had three other cases in which the same bodies were found. These scarlatinal bodies stain only by the eosin methylene blue stain and can not be demonstrated by

the hemotoxylin or Borrell stain. Dr. Mallory had tried a number of other stains, as had Dr. Field. Alcohol hardened sections could be used, but alcohol is not a good fixative. Zenker's giving by far the best results. Dr. Mallory had stated that the bodies first appeared in the epithelial cells of the deeper layers, as small rosette like bodies connected by more delicate lighter blue staining material with a central point. In a later stage they break up and then enlarge in the cell. These large bodies were homogeneous, staining with methylene blue, and as they increased they became reticular. They were both intra- and extra-cellular and the latter showed what appeared to be ameboid motion. The determination of the exact pathological significance of these bodies is a very difficult matter and it cannot be proved on purely morphological grounds. In sections of corneas treated with dipththeria toxin, Dr. Ewing has shown that we can get keratinization which simulates the ameboid forms, It is supposed that they enter the cells and there become larger, thickening, and giving out a smaller reticular structure; then they enlarge gradually, become more dense, homogeneous, shrink, and finally form rosettes again; how many times they pass through the cycle Dr. Mallory was not prepared to say. He had found them in four cases in the skin, in the epithelial lymph sinuses of the tongue in one. Dr. Field had examined skin from the groin of five scarlet fever patients dead from the disease, two patients dying on the third day of the rash, one on the second day, two on the fourth day. In all five cases he had found these bodies. Dr. Mallory had examined them and had agreed that they were similar to those he found. Dr. Field had also examined the skin of four patients living at the time the skin was taken; in one case on the first day, in two cases on the

fifth day, and in one on the sixth day of disease. In none had he been able to demonstrate the presence of these bodies. The skin had been taken from the thorax and not from the thigh. He had been able to find reticular bodies in the cells, a few outside of cells and a few he was not sure of, and he was not sure at all what they were in the lymph sinuses. He had been unable to find any of Dr. Mallory's rosettes. In Dr. Field's opinion these bodies were much more definite and delicate in structure than any degeneration he had seen. They stained in a manner similar to the malarial parasite, but not so intensely. They do not take up methylene blue so intensely. What their origin is, or how they pass into the skin, Dr. Field did not know, except that from the appearance of the skin, they seemed to have entered by way of the lymphatics. He thought the significance of the bodies might be determined from the morphological status in time, but he thought the problem would require a good deal of control work on sections of skin from a variety of conditions. He did not accept them as protozoa or degeneration, and at present was a complete agnostic on the subject.

Discussion.

Dr. I. Levin said that ten years ago he did a great deal of work on the protozoa of cancer. When he turned to this study two facts struck him. First, that the so-called protozoa of cancer resembled to a great extent the formations found in small-pox or scarlet fever, so that without the inscription he could hardly distinguish one from the other. Second, he found in his study of the protozoa of cancer, that it was easiest to find these pictures in skin cancer-ulcus rodens. This might be caused either by the

fact that the epithelium of the epidermis undergoes sooner different processes of degeneration or that the protozoa penetrate the skin more easily than the other tissues. It rather spoke against the parasitic theory that the ulcus rodens is comparatively a benign cancer. In this question as in a number of others in pathology, the morphologist was powerless to solve the problem unaided. Until such time as experimental pathology could find some way to get nearer to the physiology of the parasite, we would spend a great deal of time and energy and say at the end: "I do not know whether it is a degeneration or a protozoon."

Dr. Field said that he thought Dr. Levin had put it too strongly in saying the student could not decide the question. Dr. Field thought that with control work the question could be decided. The malarial parasite had been found to be an etiological factor in malaria. Had it been impossible to discover it in the blood it would have been found to be very characteristic in the stained sections. He thought anyone who had looked at Dr. Homer Wright's specimens from the Delhi boil, in which he demonstrated the parasite in that disease, would agree that these questions could be decided in that way. He thought the question under discussion could be decided in the same way if studied long enough. The bodies were striking and particularly well differentiated. Dr. Field thought, however, that it would require a great deal of work on numerous skin lesions or internal organs of scarlet fever patients before the nature of the lesion could be decided, but he did not despair of in time being able to help in the matter.

A CASE SIMULATING HODGKIN'S DISEASE.

HARLOW BROOKS, M.D.

Dr. Brooks showed specimes from a case simulating Hodgkin's disease. The patient was a boy about twelve years of age. He had been at the Montefiori Home for three years. The history of the case dated back to April, 1901. The onset of the disease had been sudden with vomiting, loss of consciousness and pains in the abdomen. The patient was prepared for an operation for appendicitis; but on palpation over the abdomen the tumor formation was found and it was decided not to operate. In two weeks the boy improved and was taken home. Later, the abdomen began to grow and he stooped forward, complaining of pain on the left side in the region of the bladder. Afterward some diminution in the size of the abdomen took place but the boy still walked in a peculiar position, the thigh on the left side held rigid and the body stooped over to relax the muscles of that thigh. Subjectively he complained of nothing. Physical examination showed a poorly developed, poorly nourished, dark complexioned boy, no skeletal deformities, no skin lesions. Thoracic organs negative; veins anemic. The glands of the groin were palpable but at first without perceptible enlargement. He fattened up considerably after fours years in the hospital but the abdominal circumference increased. Nothing of any importance developed in the thoracic condition. An exploratory laparotomy was performed, and, commencing at the median line in the abdomen, a glandular growth was found. A nodule was removed for microscopic examination and a diagnosis of probable peritoneal endothelioma was made. Frequent blood examinations were made but no leucocytosis was found up to shortly before

death when complicating lesions caused the development of leucocytosis. Differential leucocyte counts had shown the relative percentages to be approximately normal. The treatment of the case was unimportant and without effect on the course of the disease. Dr. Brooks showed the organs from the case.

The heart was negative. The lungs showed intralobular pleurisy; their tissue was salmon-colored, very firm, and showed microscopic changes like those of the ordinary cardiac lung. Dr. Brooks had thought at the time of autopsy that an infiltration of lymphocytes into the lung was present, but microscopically this was not substantiated. A moderate increase in fibrous connective tissue was found; otherwise the lung was like that in ordinary cardiac cases. The glands of the neck were enlarged to a considerable degree. The precardial lymph nodes were moderately enlarged; those at the bifurcation were markedly so. They were very firm and light pink in color. Dr. Brooks had described the liver in his post-mortem examination as showing lymphoid infiltration, but this was disproven microscopically. The spleen was enormously enlarged. There were whitish nodes present in it which Dr. Brooks took to be hyperthrophic Malpighian bodies. Microscopically these were found to be made up of dense masses of fibroblasts and connective tissue fibrils, and in places amyloid degeneration had taken place. On opening the stomach Dr. Brooks had found whitish patches which he took to be infiltration with lymphocytes, but which proved to be areas of fibrosis. Microscopically the kidneys were also found free from leucocytic infiltration.

The main abdominal tumor was found to be made up of the greatly hypertrophied retroperitoneal lymph nodes which although they still preserved their general

contour had grown so in size that some of them measured as much as ten cm. in diamter. Their tissue was found to be very firm and no areas of necrosis could be made out, though in certain places the tissue was slightly yellowish. The capsules of the glands seemed to be intact throughout and although they were firmly attached to the surrounding structures and to the periosteum of the vertebræ, infiltration outside the nodes could be made out nowhere. Microscopically the structure of these nodes varied greatly though all were characterized by replacement of the normal lymphoid cells by masses of proliferating fibroblasts and connective tissue tendrils. Some of the nodes showed between this connective tissue network very numerous giant cells, having, however, no definite arrangement; nothing like tubercle formation was to be made out in any of the sections. A few of the lesser enlarged nodes showed apparently a simple hyperplasia of the lymphoid cells, such as one sees in the nodes of typical cases of Hodgkin's disease. In the left iliac fossa was a great mass of enlarged glands measuring 13 by 8 cm. The microscopic structure was found to vary in the degree of fibrosis as did the other nodes previously described. A few of the nodes here on gross examination showed a curious yellow pigment deposit, like a sprinkling of the surface with sulphur. Microscopically this pigment was found to be deposited in the cytoplasm of the lymphoid and connective tissue cells. Although these glands practically filled the iliac fossa on this side and by their pressure and that of the corresponding enlarged inguinal lymph nodes caused the flexed position of the left thigh, in no place had the growth invaded outside tissues, not even the periosteum of the iliac bone to which the mass was firmly attached.

Dr. Brooks thought the diagnosis of the case lay

between sarcoma and Hodgkin's disease, in which the hyperplastic lymph cells had been replaced by fibrous connective tissue. He had never heard of nor seen a case in which replacement was so complete as in this instance, or one in which the picture presented by different glands varied so markedly. There was no typical fibrillar arrangement leading to the diagnosis of syphilis and this diagnosis would have suggested some disease of the bone—some skin manifestations, some syphilitic symptoms—none of which were present in this case. With syphilitic glands so large as these necrosis would probably have been found somewhere in the nodes. Tuberculosis had, of course, been considered, and Dr. Brooks thought excluded, because in this enormous mass of tissue one would expect to find something fairly characteristic of it, were it tubercular. Sarcoma might be diagnosed, in which case it must be classed as a fibrosarcoma; but it is contrary to our knowledge of this growth to have it limited to the lymphoid tissues, as in this case. If sarcoma, we would have expected the tumor cells to have infiltrated outside of the Malpighian bodies, in the spleen, for instance. We would also have expected the growth to burst out through the capsule of the glands and to have invaded the surrounding structures. Dr. Brooks thought it a case of Hodgkin's disease in which there was replacement of the hyperplastic lymph tissue by the connective tissue hyperplasia which is found so commonly in chronic inflammatory processes.

Discussion.

Dr. Ewing said he had examined the sections from Dr. Brooks' case and believed that both the histology and the gross appearance left no room to doubt that the case was one of Hodgkin's disease. Compared with

others the tissue in this case contained many more giant cells than is usual and these cells gave the section somewhat of a sarcomatous appearance. This histology had been regarded for many years in Vienna as pathognomonic of Hodgkin's disease, and although it seemed to be less frequent in New York, it had recently been described anew in a series of cases by a Baltimore writer.

In some cases of *constitutio lymphatica* he had seen a good many giant cells in the hyperplastic lymph nodes and would like to know if in this case there were any of the signs of the lymphatic constitution, such as rickets or persistent thymus.

The absence of necrosis while characteristic of this type of Hodgkin's disease did not, he believed, rule out a tuberculous origin, which could only be attempted by inoculations of large amounts of material. On the histology alone he did not think it possible to exclude tuberculosis.

Dr. Brill said that the doubt expressed by Dr. Brooks as to the pathological nature of this case was but another indication of our lack of knowledge of the pathology of Hodgkin's disease. While the disease represents a definite clinical entity, its pathology presents a number of various and not allied or identical conditions. Clinically, the disease presents anemia without marked qualitative changes in the red and white cells of the blood, enlargement of the lymph nodes, and in almost all cases, more or less increase in the size of the spleen, accompanying progressive weakness, and finally, almost invariably a fatal issue in two or three years or less. When the disease is considered histologically and pathologically, it represents this clinical picture arising from a lymphoid hyperplasia, not induced by tuberculosis, syphilis, rachitis, or sarcoma. It is here where our knowledge is at fault,

for cases clinically like Hodgkin's disease occur, which on pathological examination may prove to be any one of the conditions just mentioned; namely, tuberculosis, sarcoma, or syphilis.

However, because a change in some of the lymph nodes in a case of Hodgkin's disease may be due to tuberculosis of these nodes, we are not justified in assuming that that case is not one of Hodgkin's disease. for there is no reason why a coincidental infection with tuberculosis may not exist together with the original infection, if we may call Hodgkin's disease an infection, as it would seem to be from many of its aspects.

The case presented by Dr. Brooks was extremely interesting from both the clinical and the pathological points of view and Dr. Brill asked whether Dr. Brooks could say in which groups of lymph nodes enlargement was first noticed, whether the cervical, mediastinal, retroperitoneal, mesenteric or inguinal.

Dr. Brooks replied, in the retroperitoneal region.

Dr. Brill said that this was against the rule in Hodgkin's disease, where the lymph nodes of the anterior cervical or mediastinal regions were commonly first involved, considerably before the retroperitoneal or inguinal nodes. From a clinical aspect there was no reason to call this case of Dr. Brooks' anything else than one of Hodgkin's disease.

Dr. Brooks said he felt somewhat comforted at the remarks made. He said the more he studied the glands the more each one seemed to show a different picture. He could not get rid of the idea that the case was one of Hodgkin's disease, but it differed from all cases as he had seen it, and the changes in the glands were not as usually found in that disease, so far as he had been able to examine them, having studied about thirty instances. They

also differed from those of any case he had read of. In regard to status lymphaticus, the linguinal adenoids were noted as moderately enlarged in the protocol, but the tonsils were not hypertrophic, and the thymus was absent. In the detailed autopsy, only the outlines of which had been read, he did not find anything which led him to think it was status lymphaticus. The thickened areas in the walls of the stomach and gut which he had taken to be lymphoid infiltration, proved to be fibroid thickening. On the dorsum of the tongue he had found a small excavated, even-floored ulcer, which looked like a trophic ulcer. The child had never complained of this and it was only discovered post-mortem. At one side of this ulcer was a small mass of connective tissue which might have been considered a tubercle *if any other tubercles had been seen.*

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DR. OTTO H. SCHULTZE, *President.*

A CASE OF PERNICIOUS ANAEMIA,

SHOWING A MEGALOBLASTIC CRISIS FOLLOWED BY MARKED IMPROVEMENT.

D. S. D. JESSUP, M. D.

The following case of pernicious anaemia is one that for a time showed progressive changes for the worse, marked especially by increase in the number of megaloblasts. Under treatment of a few weeks there was a total disappearance of the nucleated cells and a remarkable change for the better in the condition of both the blood and the patient.

The patient was a German woman of fifty-two years of age, who came to this country when sixteen. She was married when twenty-four and had had four children and several miscarriages. Until within a year she had enjoyed good health, but had always worked hard and two years ago had nursed her husband through a long illness. Twelve months ago she began to feel run down and she said people told her that her blood was poor. She suffered from dyspnoea and constipation, and a month before coming to the Vanderbilt Clinic her feet began to swell and she became very nervous. She was living in a damp ground-floor flat, practically alone, doing all her own work up to the time of going to the hospital.

Physical Examination showed a large, rather well nourished woman with pale mucous membranes and a yellow tinge to the skin, but not the color that would suggest an advanced pernicious anaemia. In fact the diagnosis was not made until the blood was examined. There was a loud systolic murmur, loudest over the pulmonic area, and marked oedema of the legs and ankles. The urine showed a trace of albumin and hyaline casts.

For a period of ten days she was able at intervals to come to the Clinic from Harlem, and she got some relief from the bandaging of her legs and feet, but was unable to take food or keep warm. The dyspnoea increased and she grew weaker, until finally on November 11, she collapsed and had to be helped across the street to the Hospital. She had then developed a bad bronchitis with considerable temperature.

For the opportunity to make the subsequent observations I am indebted to Dr. Walter B. James, to whose service she was admitted at the Roosevelt Hospital.

Date	Hemoglobin.	Red Cells.	White Cells.	Polynuclear Neutrophiles.	Large Lymphocytes.	Small Lymphocytes.	Eosinophiles.	Mast Cells.	Megaloblasts.		Normoblasts.		Estimated Number of Megaloblasts to C.MM.	Estimated Number of Normoblasts to C.MM.
									Per Cent. to White Cells	Number seen in Count. 500 White.	Per Cent. to White Cells	Number seen in Count. 500 White.		
Oct. 30	25	1,152,000	11,000	59.	8.	30.8	2.2		3.8	21	2.4	12	342	216
Nov. 4	20	984,000	6,000 ?	50.8	10.6	36.4	2.2		5.2	27	1.6	9	312 ?	96 ?
Nov. 11	15	968,000		72.6	8.2	18.4	0.6	0.2	17.	92	2.6	16	986	150
Nov. 12			5,800	72.4	12.2	14.6	0.2	0.6	7.6	40	4.6	25	440	266
Nov. 16				77.	11.8	9.2	1.8	0.2	17.2	108	7.8	45	997	462
Nov. 27	30	1,100,000												
Nov. 28			7,800	88.6	7.	4.	0.4	0.	0.		0.			
Dec. 3	35	1,400,000	7,800											
Dec. 8			11,000	79.6	7.2	7.6	5.6		0.		0.6	3		
Dec. 19	47	3,240,000	8,000											
Jan. 7	55	3,000,000	6,500	74.6	10.	10.6	4.2	.6	0.		0.			

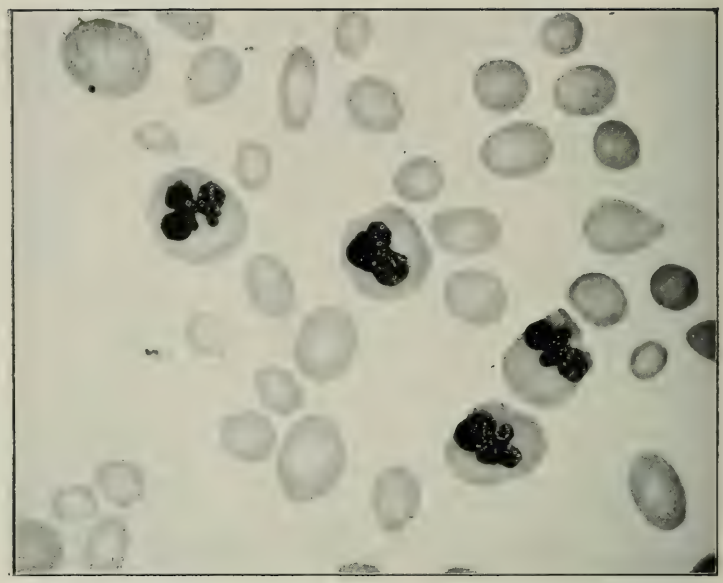

FIG 1.—Smear taken on November 11, showing group of megaloblasts.

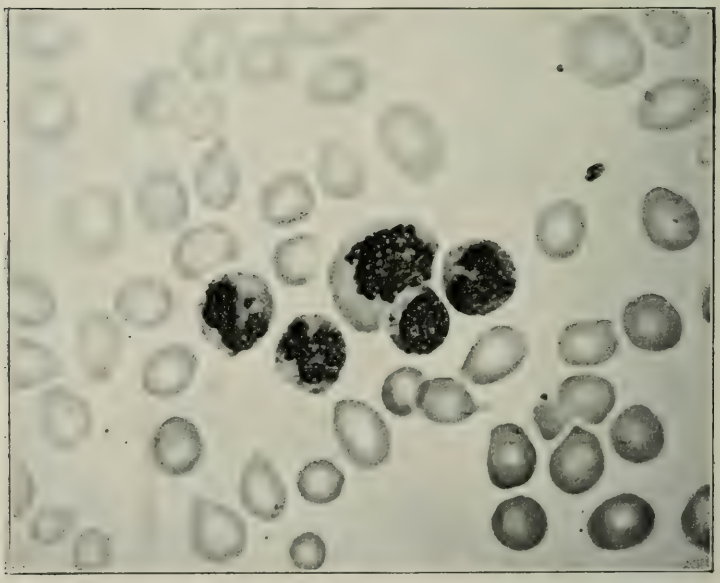

FIG. 2.—Smear also of November 11. Megaloblast showing mitosis in center of group of polynuclear neutrophiles. A lymphocyte overlies the megaloblast.

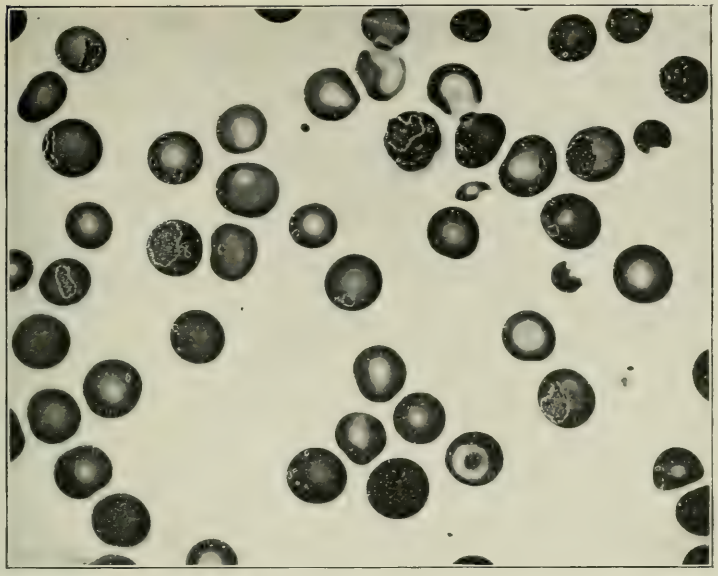

FIG. 3.—Smear taken Jannary 9. Lymphocyte in upper part of field. Nearly all the red cells are normal in size aud shape.

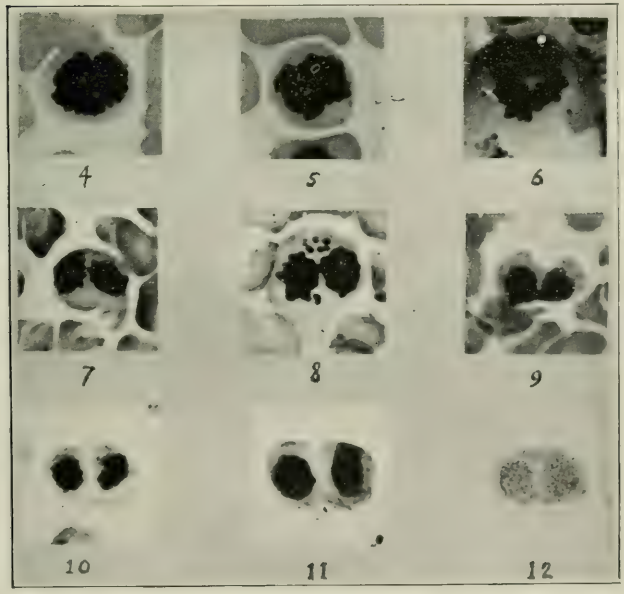

FIG. 4–12.—Megaloblasts showing various stages of mitosis.

The photographs were taken by DR. E. LEAMING, and with the exception of Fig. 12, the magnification is 1145 diameters.

Blood Examinations:

The observations on the blood were made during a period of ten weeks. The morphology was studied from smears stained with Wright's chromatin stain. This stain is the most satisfactory for pernicious anaemia, as it brings out far better than others the difference between megaloblasts with polychromatophilia and large lymphocytes. With Jenner's stain it is often very hard to differentiate between the two. Wright's stain also gives a lighter, cleaner stain to the body of the red cell, which makes it preferable also for studying chlorosis and secondary anaemias.

The number of megaloblasts in the blood on different days was estimated in two ways.

1. They were counted during a differential count of the leucocytes and their number to the cubic millimeter estimated by subtracting from the leucocyte count, inasmuch as all nucleated cells are counted in the white cell count. On those days when a differential count was made but no leucocyte count, the number of leucocytes was assumed to be about the same as the count on a nearby date.

2. In making a differential count of five hundred white cells note was made of the number of megaloblasts seen.

The number of normoblasts was estimated in the same way. Microblasts were not seen at any time. The accompanying table gives the cell counts, hemoglobin, differential count, and the number of nucleated red cells. The megaloblastic crisis appears to have occurred between November 11 and 16, when the number per cubic millimeter ran up to nine hundred and ninety-seven. This is some three hundred more than Da Costa found in the highest count in his series of twenty-nine cases. Twelve

days later careful search failed to show any nucleated cells. On December 8, three normoblasts were seen in counting the five hundred white cells, but none in the later examinations. Many megaloblasts showed mitosis. The other morphological features were those usually seen in pernicious anaemia. Megalocytes were very numerous and there was marked poikilocytosis. There was polychromatophilia of both nucleated and non-nucleated cells. At first the cells were rich in hemoglobin; later there was a more rapid increase in cell number than in hemoglobin and the index fell below 1. when the count reached 3,000,000.

The case follows the course observed in several instances where a megaloblastic crisis has been the precursor of an increased number of red cells indicating great activity of the bone marrow. The lymphocytosis is most marked before the crisis when the percentage of large and small reaches forty-seven. The eosinophiles and mast cells are within normal limits and no myelocytes were observed.

With the changes in the blood there was steady improvment in the condition of the patient. The severe attack of bronchitis developing when the blood was so impoverished (red cells below 1,000,000 and hemoglobin below 20%) had given a bad prognosis, but when this left her the dispnoea decreased, the oedema of the legs went down and for many days before leaving the hospital she was able to walk about the ward. The treatment throughout was with Fowler's solution in increasing doses up to nine drops three times a day.

The last smears of the blood were made on January 9, the day before she left the hospital. The contrast between those and the earlier smears was very great. An occasional megalocyte or poikilocyte was the only thing to suggest that the blood was not normal.

THE ACTION OF DYSENTERY BACILLI ON NUTROSE MEDIA.[1]

HERMAN SCHWARZ, M.D.

Dr. Schwarz said that in the following short demonstration he would attempt to show the action of the varieties of dysentery bacilli upon nutrose media. As was probably known to those present, nutrose is prepared from fresh cow's milk, and is a substance given to invalids, babies, etc. It is the soluble compound of casein. Nutrose was first used by Barsiekow[2] and then by Klopstock[3], who used it for differentiating typhoid and coli. It forms a part of the Drigalski and Conradi medium, and Doerr[4] has used it in differentiating the varieties of dysentery bacilli.

With this medium and a number of the strains of the bacilli at the laboratory, Dr. Schwarz had observed the differences in the various sugars, using both solid and fluid media.

The fluid medium was made with 1 per cent. of nutrose, 0.5 per cent. sodium chloride, and water. This was put in the sterilizer for one hour and then filtered. A standard solution of litmus was always used, that of Kubel and Tieman prepared by Kahlbaum.

The solid medium was made by the addition of 1.5 or 2 per cent. of agar to the above. The acidity of this medium was about 0.2 per cent. The sugars were chemically pure as far as could be obtained. The sugars were first dissolved in water, then sterilized for ten minutes on

1 Received for publication October 7, 1904.

2 *Wien. klin. Rundschau, 1900.*

3 *Berl. klin. Woch,, 1902.*

t., 1903.

two successive days, and then added to the medium in various proportions.

The types of the dysentery bacilli used, were the Shiga type from Japan, Flexner-Baltimore type, Mt. Desert type, and the Brooklyn or Coney Island type.

In order to explain how these bacilli act upon nutrose the action of the Bacillus coli upon nutrose was demonstrated, showing the complete coagulation and reddening of the media with glucose and a similar reaction when lactose, maltose, etc., were added.

Fluid nutrose media without addition of sugar; made by taking 1 per cent. nutrose, 0.5 per cent. sodium chloride, and 1.5 per cent. litmus in water.

Shiga after 24 days remained unchanged, Flexner, unchanged. Mt. Desert, unchanged. Brooklyn, unchanged.

Dr. Schwarz added that the Bacilli coli does not produce any acid in this medium, nor is there gas produced in fermentation tubes. The fluid nutrose solution contains very little sugar, as far as the production of gas aud acidity is concerned, as shown by the action of the colon bacillus.

Dextrose medium; made by adding the sterilized dextrose to the amount of 1 per cent. to the sterilized nutrose solution, and sterilizing for ten minutes for two successive days. Numerous controls were always added and when one control was contaminated the entire series was thrown out.

Shiga, acid in 24 hours, clear, no cloudiness or coagulation; the same in 48 hours, and after 13 days. Flexner, acid in 24 hours, no cloudiness; 48 hours, acid, no cloudiness; but at times various cultures were cloudy or even coagulated after 48 hours. Mt. Desert type, as Flexner. Brooklyn type, as Shiga.

Lactose medium. All groups show absolutely no change during 40 days growth.

Saccharose medium, 1 per cent. Shiga, 24 hours up to 12 days, unchanged. Flexner, 24 hours slightly acid; the same after 12 days. Mt. Desert, unchanged after 12 days. Brooklyn type very faintly acid after 12 days. The acidity in the Flexner and Brooklyn types was variable; entire series going 14 days did not show any acidity. The use of 2 per cent. saccharose showed the same changes; the change in Flexner and Brooklyn being perhaps slightly more marked. There was no difference in this result when reaction of the nutrose was altered from 0.1 per cent. to 0.5 per cent. acidity. A greater acidity than 0.5 per cent. usually produced coagulation of the nutrose.

One per cent. maltose-nutrose medium. Shiga, 24 hours unchanged; after 14 days unchanged; on the 21st day slight acidity produced. Flexner, 24 hours, acid, clear, no coagulation; 72 hours, acid, slightly cloudy, no coagulation; the same after 21 days, no coagulation. Mt. Desert, unchanged up to 21 days. Brooklyn, 24 hours, unchanged; unchanged after 14 days; slightly acid on 21st day. The acidity with Flexner is very marked in 24 hours, and very constant; and the acidity in Shiga and Brooklyn is very slight and very slow in appearing (21 days). This late appearance of the acidity is very constant, using 1 per cent. of maltose. With 2 per cent. of maltose, acidity in Shiga and Brooklyn type took 8 days to develop.

One per cent. levulose-nutrose medium. Shiga, Flexner, Mt. Desert, and Brooklyn, all produced marked acidity and cloudiness; Flexner and Mt. Desert caused coagulation; Shiga and Brooklyn caused acidity and cloudiness, but no coagulation.

Mannit-nutrose medium. The reaction here was very constant, after 14 days Shiga showed no change. Flexner acid in 24 hours, cloudy and slight coagulation in 72 hours; absolutely coagulated in 7 days. Mt. Desert, acid in 24 hours; 48 hours acid and coagulated; 7 days likewise. Brooklyn, 24 hours, acid, clear, no cloudiness; the same in 7 days. Here Shiga is not acted upon; Mt. Desert is most active; Flexner-Baltimore next, and last, Brooklyn.

Dextrine medium. The results with dextrine have been so variable, owing to the impurity of the dextrine, that no conclusions have been drawn.

This summed up the work on fluid media.

The solid media used were plain nutrose litmus agar, mannit nutrose litmus agar, maltose litmus agar, and lactose nutrose litmus agar.

Plain nutrose-litmus agar, stick culture. Shiga, 24 hours slightly acid, as far as stick goes; 48 hours, acidity three-fourths of the tube; 72 hours, the upper one-fourth is alkaline; 96 hours alkalinity increasing, the entire tube never being red; 110 hours acidity diminishing, alkalinity increasing; 10 days, almost all blue. Flexner-Baltimore, 24 hours, acid as far as the stick; 48 hours, entire tube acid; 72 hours, one-fourth inch from the top is alkaline; 96 hours, three-fourths from the top all blue; 10 days, almost blue. Mt. Desert as in Shiga, the entire tube never being red. Brooklyn type likewise. There was thus an acidity of the entire tube of Flexner, and partial acidity of the others, and then a gradually increasing alkalinity beginning from the top of the tube. It must be remembered that with plain nutrose litmus solution there was no acidity produced, as far as the change in the color of the medium would show.

Lactose-nutrose agar, stick culture. Shiga, 24 hours,

acid, the upper one-eighth inch is alkaline; 48 hours, acidity decreasing, alkalinity increasing; 96 hours alkalinity increasing markedly; 6 days, tubes entirely blue. Plates made of lactose-nutrose agar (both surface and deep plates) never showed any acidity. Flexner, as Shiga. Mt. Desert and Brooklyn, the same. Here there was a beginning acidity followed by an alkalinity in the tubes, but no acidity in the plates.

Mannit-nutrose agar, stick culture. Shiga, 24 hours, acid, upper one-eighth inch alkaline, but alkalinity gradually increased until after 7 days, when the tube was like control. Both surface and deep plates were alkaline from the beginning. Flexner-Baltimore, acid in 24 hours almost through the entire tube; in 48 hours, entire tube acid, remaining so to 17 days, the last time of observation. Surface and deep plates acid from beginning. Mt. Desert and Brooklyn as Flexner. This showed a decided difference between Shiga and the others.

Maltose-litmus nutrose agar, stick culture. Shiga, acid in 24 hours, but upper one-eighth inch alkaline; in 48 hours, alkalinity slightly increased; after 144 hours the tube was but slightly different from the control. Surface and deep plates were alkaline from the start. Flexner, 24 hours, acid to the top of the tube; 144 hours, acid throughout the entire tube. Surface and deep plates acid. Mt. Desert as Shiga, only slightly less marked. Surface and deep plates alkaline from the start. Brooklyn same as Shiga.

Just why acidity should be produced in the solid media and not in the fluid, Dr. Schwarz said he could not entirely explain. He had tried to find sugar in the nutrose by fermentation with yeast, by acid production with coli, and by chemical tests such as Fehling's, Nylander's, etc., after conversion with acid of any sugar

that might be present, but he had failed to get any reaction. The bacilli were grown in the fluid media under anærobic conditions, and yet no acid was produced.

This completed the work that Dr. Schwarz had done on nutrose so far. He believed that it was a useful adjunct in helping to differentiate these varieties of bacilli. Seeing that the color was retained so well in the coagulum, due to the growth of the colon bacillus in this medium, and that none of the dysentery bacilli produced acid on the lactose agar plates, Dr. Schwarz conceived the idea of making a lactose nutrose litmus agar, and using it as a differentiation between coli and the dysentery bacilli. This medium is really a modification of Drigalski and Conradi's. The acidity of this medium, contrary to the above anthors, was made 0.2 per cent. acid instead of 0.2 per cent. alkali.

It was made up as follows: 1 per cent. nutrose; 1 per cent. lactose; 0.5 per salt; 1.5 per cent. agar; and 10 per cent. Kahlbaum's litmus, after Kubel and Tieman's formula. The nutrose with the salt was first dissolved in water in the sterilizer for one hour; then the agar was added, and colored with an egg. The sugar was then added to the extent of 1 per cent., the reaction brought to 0.2 per cent., and the litmus added. It was then tubed and sterilized on three successive days. This medium was used only with surface plates. The coli produced red colonies, with their color fairly well retained, very little spreading into the surrounding medium. Dysentery bacilli were blue. This medium has the advantage of being very easily prepared and fairly reliable. It may be considered a useful adjunct in isolating dysentery bacilli from the stools.

Discussion.

Dr. E. Libman said the attempt to differentiate colon, para-typhoid and typhoid bacilli by means of precipitation with sugar media was of interest to him personally because in 1901, in a paper dealing with the growth of bacteria on sugar media, he had suggested the possibility of using such methods as those which have been introduced during the last few years. He appreciated at the time that it was difficult to avoid diffusion of the acid. This was particularly true if many streptococci were present (especially in stool examinations). He thought it would be wise to keep trying these methods. However, the recent attempts at finding typhoid bacilli by plating on special media after getting rid of some of the other organisms (for instance, by the addition of caffein) would certainly be more effectual if they were successful.

From Dr. Schwarz' demonstration of the growth of the various bacilli in the tubes containing lactose and nutrose it was evident that there was something in the media that was not free from carbohydrate; otherwise in a medium containing agar, nutrose and lactose, it ought to be impossible for any acid to be produced by the growth of the dysentery, typhoid, or paratyphoid bacilli. He personally had never succeeded in getting growth in the branch of the Smith tube with any of these organisms.

Dr. Libman indicated that it was necessary to observe all the tests not only after 24 hours, but also after 8 and 12 hours, for sometimes the slight amount of acid produced by the growth of bacteria would be replaced by alkali at the end of 24 to 30 hours. This would, of course, only happen when the growth was on the surface

of the plate or in bouillon tubes, or in the bulb of the fermentation tube.

Dr. Schwarz said that the fermentation of the lactose (?) in the lactose agar stick cultures bothered him a good deal. He could not explain it for he had tried to find the presence of sugar in the nutrose as stated in the demonstration. He tried the agar without nutrose but could not find any sugar. Fermentation tests were also negative. He agreed with Dr. Libman that there must be something in the nutrose, but just what he could not say.

A DISCUSSION ON SEVERAL NEW POINTS CONCERNING THE THEORY AND PRACTICE OF IMMUNITY.*

PROFESSOR A. WASSERMANN, M.D.,

BERLIN.

I am glad, gentlemen, to utilize the opportunity afforded by this visit to the United States to present to you a few facts concerning the theory and practice of immunity. Before such an audience as this it will be unnecessary to enter deeply into the fundamental ideas of the doctrine of immunity, nor into the specific therapy so intimately connected therewith. I shall therefore confine myself above all to the experiments and studies which I, my co-workers, and my pupils, have recently made in the Institute for Infectious Diseases, Berlin.

You are all aware, gentlemen, that the investigations in immunity during the past few years have been domi-

* Reprinted by permission from the *New York Med. Jour. and Phila. Med. Jour.*, *Oct. 15, 1904.*

nated by the so called side chain theory formulated by Ehrlich. You probably also know that this theory has been the subject of considerable discussion, many authors having brought forward experiments to support the theory, other authors, on the contrary, experiments to refute the same.

The theory itself is extremely simple and seeks to explain the manner in which the protective substances appear in the blood in immunity. Ehrlich began by observing that of the many poisonous substances known to us, only a comparatively small number existed against which we could truly immunize, *i. e.*, obtain specific antibodies in the blood serum of the immunized organism. Let us look at two poisons which are very similar in their physiological action, for example, strychnine and tetanus poison, both of which excite spasms through the central nervous system. It is really curious that the injection of one, strychnine, produces no antibody whatever in the serum, while the injection of the other, the tetanus poison, causes the formation of the specific tetanus antitoxine. Ehrlich says that this is because these substances enter into entirely different relations with the cells of the living organism. The one substance, strychnine, merely enters into a loose combination with the cells of the central nervous system, so that it can again be abstracted from these cells by all kinds of solvents, *e. g.*, by shaking with ether or chloroform. The combination, therefore, is a kind of solid solution, such as Witt has shown for the staining with aniline dyes. The tetanus poison, on the contrary, Ehrlich says, is firmly bound to the cell; it enters the cell itself, becoming a chemical part of the same, so that it cannot again be abstracted from the cell by solvent agents. He compares this process to the assimilation of nutrient substances.

Hence the difference between those two substances could be likened to that between saccharin and sugar. Both substances taste sweet, but, despite this similarity in one of their physiological actions, they behave very differently toward the cells of the organism. Saccharin simply passes through the organism without entering into a firm combination, *i. e.*, without being assimilated, and is therefore no food. Its sweetening action is due to a mere contact effect on the cells sensitive to taste. Sugar, on the contrary, is actually bound by the cells, assimilated and burnt, so that it is a true food. Ehrlich says that the first requirement for every substance against which we can obtain a specific serum must be its power to enter into such a combination with certain particular cells in the living organism. The substance must possess a definite chemical affinity for certain parts of the organism. Hence in each substance against which we can specifically immunize, Ehrlich distinguishes a group which effects the specific binding to certain cells, the *haptophore group*. Corresponding to this is a group on the cell of the living organism, the *receptor group*, with which the haptophore group combines. The latter is entirely distinct from that part of the substance which exerts the physiological or pathological effect, in toxines, for example, from the group which is the carrier of the poisonous action, the so called *toxophore group*, or in ferments, from the group which exerts the ferment action, the *zymophore group*. Both groups, haptophore and functional, are independent of each other, and their separate presence can easily be demonstrated because the functional group, *e. g.*, in poisonous toxines the toxophore group, is more readily destroyed by heat than the haptophore group. Thus by heating a toxine for some time to 140° to 158° F. substances will be obtained

which are no longer poisonous, but which still possess the binding, haptophore, group. In the case of toxines such substances are called *toxoids*. Ehrlich further stated that the finer mechanism of the formation of specific substances was this, that the haptophore group was bound to the receptor of the living organism owing to a specific affinity. As a result of this the receptor is lost to the living organism, disposed of, and a biological law formulated by Weigert now comes into action, the law of supercompensation. That is, the organism seeks to replace this defect, but in doing so not merely replaces the receptors in question, but, according to Weigert, produces more of them than were previously present. The conditions are somewhat like those seen in the callus after a fracture, in which the organism likewise does not produce just the amount of bone previously present; there is always an overproduction.

In this way, Ehrlich says, such a large number of receptors are produced by the organism that these become too much for the same; they are then thrust off into the blood as ballast, and these free receptors circulating in the blood constitute the specific antibodies. Ehrlich therefore believes that the specific antibodies in the serum are nothing else than receptors for which the substance employed in immunization possesses specific affinity. Hence the same substance which, so long as it remains in the organ, atracts the toxine and makes it possible for that to exert its poisonous action on the organ, this same substance acts as a protection when it circulates outside of the organ; for then it satisfies the affinity of the poison's haptophore group while still in the blood, preventing the poison molecule from acting on the organ itself.

In the formation of the specific antibodies we must therefore distinguish three stages:

1. The binding of the haptophore group to the receptor.

2. The increased production of the receptors following this binding.

3. The thrusting off of these increased receptors into the blood.

A great deal of what I have just spoken to you has already been confirmed experimentally. For example, the presence of the separate groups and of the haptophore group; the existence of specific binding properties for certain cells, in all substances with which we can immunize; and much more. These matters, which go back several years, I shall not here discuss, but shall pass at once to several new experiments of which I made mention at the outset.

Until recently there was a gap in our experimental demonstrations in this respect, that the existence of the second stage, the increased production of receptors following the binding of the haptophore group to the receptor, had not been experimentally demonstrated. I believe that I and my fellow worker Bruck have now been successful in this.

We employed a tetanus poison which I had kept since 1896. This poison was originally very poisonous. In the course of years, however, owing to the damaging influence of this long standing, that is, owing to the action of light, the oxygen of the air, etc., it had become so weak that it was no longer toxic at all. I was able to give a guinea pig one c.c. without producing tetanus. Nevertheless the haptophore group had remained intact, as could readily be proved, for this non-poisonous tetanus toxine was still able to bind tetanus antitoxine, *i. e.*, thrust off receptors. We are therefore dealing with a completely non-poisonous tetanus toxoid. When I

injected rabbits with this non-poisonous tetanus toxoid in increasing doses, and then examined the blood serum of the animal, I found *not a trace of tetanus antitoxine.* The absence of antitoxine in this experiment could have either of two causes:

1. It might be that the toxoid no longer produced any physiological effect whatever in the organism; or

2. Although it still caused an increase in the receptors, these increased receptors remained in the organism (sessile), and were not thrust off into the blood.

In order to decide this question we first determined the exact quantity of fresh tetanus toxine which constituted a fatal dose for guinea pigs. If, now, the action of the tetanus toxoid was such that in the living organism it was *not* bound to the receptors, it should be possible to prove this experimentally.

Our line of reasoning was as follows: Were we to inject first the toxoid, and shortly after, say, in one to two hours, the fresh toxine, we should in such an animal have to increase the fatal dose, *i. e.*, we should require more tetanus toxine to kill this animal than a normal one, because owing to the previous toxoid injection part of the cells susceptible to tetanus toxine had already been occupied. Provided Ehrlich's theory was correct, so that this binding of the toxoid really occurred, the conditions should be entirely different when, instead of injecting the toxine shortly after the toxoid, I waited somewhat longer, one to three days, and then injected the fresh tetanus toxine. For in that case Weigert's law should come into play and the receptors have commenced to increase in number, *i. e.*, the organ would now possess more sensitive groups than before. This would have to manifest itself in such fashion that in contrast to the first experiment the fatal dose of fresh tetanus toxine

could now be decreased; in other words, a small dose would now tetanize the animal in a shorter time.

As a matter of fact,, the experiments yielded results which were exactly like those deduced theoretically as just described. I injected a guinea pig with some of the non-poisonous toxoid, and then, one hour later, with the tetanus toxine. I found that much more toxine was required to kill this animal than a normal guinea pig of equal size. If, on the contrary, I waited one to three days, it was found that then a dose of tetanus toxine which would not even tetanize a normal guinea pig was sufficient to kill this one.

I believe that if we keep this series of experiments in mind we shall find that it furnishes for the first time experimental proof of the existence of the three stages demanded by Ehrlich's theory. The first stage, the binding of the haptophore group, we have demonstrated with our toxoid; the second stage, the increased production of receptors is demonstrated by the second series of experiments just described; the third stage is the thrusting off of the receptors. Close attention to these experiments will have shown you that with our completely non-poisonous toxoid we did not effect this thrusting of at all. The serum of the rabbit treated with this toxoid contained no antitoxine whatever. Hence it follows that the thrusting off of the receptors into the blood serum cannot be a function solely of the haptophore group, but that something additional is required. This "something," which we may term a stimulus, is a function of the toxophore group, as I have been able to show experimentally. Because of these experiments I conceive the mechanism of the binding of the specific antibodies in the serum to be as follows: 1. The haptophore group is bound by the receptor. 2. The

consequence of this binding is a proliferation of the receptors. In this stage, however, they are still attached to the organ. 3. In order that they be thrust off, a certain stimulus is required. The haptophore group is incapable of exerting this so that in the case of tetanus toxine, this is exerted by the toxophore group.

So much, gentlemen, for the mechanism of the formation of these substances, especially of the antitoxines.

Another point, not only of theoretical but also of practical interest, and one which I shall therefore discuss, is that concerning the action of antitoxines on toxines, a question on which really the entire system of serum therapy rests. As you may know, just this action of antitoxine has recently been the subject of extended scientific investigations from the standpoint of physical chemistry. Particularly Arrhenius and Madsen have tried to explain these relations purely according to the laws of that science.

Together with my coworker Bruck I have been able to show, especially for tetanus toxine and its corresponding antitoxine, that this action also can be divided into several stages, entirely in harmony with the views of Ehrlich. The first stage consists in a loose association of the two substances. This is followed by a true chemical union, which becomes firmer as time goes on. The manner in which we are able to demonstrate this in the living organism—and only in this can we obtain results of value to the physician—is seen in the following experiment.

We know from the researches of Meyer and Ransom that tetanus *poison* is not absorbed by way of the blood and lymph channels, but reaches the central nervous system by way of the nerves. Following its injection the tetanus poison ascends in the axis cylinder of the

nerves to the motor cells. The tetanus *antitoxine* unlike the toxine is not a neurotropic substance, but always follows the blood and lymph channels. In adrenalin, discovered by Takamine, we possess a substance which is able strongly to contract the capillaries and thus to block the blood absorption path of a particular area. Proceeding from these facts, I devised the following experiment: Tetanus toxine and antitoxine were mixed in such proportions that the mixture was entirely innocuous to animals, *i. e.*, it was neutral. If this mixture was injected into the hind paw of a guinea pig no tetanus developed. When however, I injected some adrenalin into the hind paw of a similar sized guinea pig, and waited a few minutes until the capillaries had contracted, and than injected my mixture of tetanus toxine and antitoxine, typical tetanus was produced. What happened was this, that the channel of absorption for the tetanus antitoxine had been blocked, while that for the toxine, the nerve path, was open. The toxine had therefore torn loose from its combination and followed its course to the central nervous system, where it produced tetanus.

This experiment, however, succeeds only within a certain period, *i. e.*, not over an hour after mixing the toxine and antitoxine. If we wait a longer time, say, three or four hours, it will be found that even in the adrenalin animal no tetanus is produced, because by this time the combination, previously a loose one, is so firm that the substances can no longer be torn apart. This union can be hastened by employing more tetanus antitoxine, for with an excess of antitoxine even after only half an hour it is impossible by means of adrenalin to loosen the tetanus toxine. This experiment, therefore shows that the tetanus toxine-antitoxine combination is at first a loose one, and that the affinity becomes firmer

and firmer with time. It also shows the possibility of increasing this affinity by means of large doses of antitoxine, a point of considerable practical value in serum therapy.

This increase in the firmness of the combination and these several stages, both of which can be demonstrated in the living organism as just described, are not easily reconciled with the views of Arrhenius and Madsen. However, I shall not enter further into these purely technical and theoretical questions, but rather turn to the more practical results of the investigations in immunity.

In this connection I shall first speak of the finer structure of the antibodies in the serum, a point of great practical importance, as you will shortly see. According to Ehrlich's theory, as you may recall, every immunizing group in a substance corresponds to a counter group of the fitting receptors in the organism. Now, we can demonstrate that the body of certain bacteria, *e. g.*, *streptococci* or *Bacterium coli*, is not a homogeneous mass, but is made up of various molecules which differ biologically from one another. Conforming to this, the antibody which appears in a serum is made up of the sum of the single receptors which correspond to these partial elements in the bacterial body. These separate groups are called "partial groups." A serum therefore consists of the partial groups. An immune serum therefore consists of the partial groups which correspond to the separate partial elements of the bacterial body. We are further able to show that these partial elements in one and the same bacterial species are not the same for all the bacteria of that species. Thus one culture of streptococci or *Bacterium coli* may have a few partial elements which differ from those of another culture. What is the consequence of this? The consequence will be that

when we immunize with a culture *a* of such bacteria we shall obtain a serum which acts completely on this culture, for in this serum all the partial elements present in culture *a* are represented. If, however, we employ culture *b*, *c*, or *d*, which perhaps possess other partial elements, we shall find that the serum does not completely affect these cultures. As already stated, such a condition of things is met with in streptococci, in hog cholera, and in other bacteria, and is therefore of considerable practical importance. It is because of this fact that such a serum acts only in a limited number of cases and not in others. In order to overcome this difficulty in these bacterial species we have no choice but to make sera, not by means of *one* culture, but by means of a number of different cultures of the same species. The result of this will be that, corresponding to the various partial elements in these different cultures, we shall obtain a serum containing a large number of the partial groups. Such a serum will then exert a specific action on a large number of different cultures.

In other words, the development and the closer analysis of the problem of immunity, especially during the past few years, have shown us that we must make use, more than heretofore, of so called *polyvalent* sera. In passing I may say that the term "polyvalent" seems unfortunately chosen, and therefore, in order to indicate that such a serum differs from a monovalent serum (one derived by means of a single culture), I always use the term *multipartial*. Such a serum, then, contains a number of partial elements. In the serum therapy of streptococci, of *Bacterium coli*, of the excitants of certain hæmorrhagic septicæmias, and, as we shall at once see, to a certain extent also in diphtheria, I consider the production of such multipartial sera absolutely necessary in

practice. Owing to these partial groups also, a serum, *e. g.*, typhoid serum, can specifically affect a closely allied species of bacterium, like *Bacterium coli*, for example. For it is known that closely related species of bacteria, such as typhoid and *Bacterium coli*, possess certain partial groups in common, and a serum is thus produced which to a certain extent acts on both species. This constitutes what is familiar to you as the "group reaction".

This principle of the production of multipartial sera is also of considerable importance to species to which heretofore it was not thought to apply. This is illustrated by a new diphtheria serum which I described in a short communication about a year and a half ago.

As you are all aware, we have thus far in practice made use only of Behring's antitoxic diphtheria serum, *i. e.*, one which acts only on the toxine of the diphtheria bacilli, but not on the bacilli themselves nor on the bacterial cell substance. I have recently called attention to the fact that it is possible to obtain a serum which acts on the diphtheria bacilli themselves. Such a serum is obtained when, under certain conditions, diphtheria bacilli in large numbers are inoclulated into animals. This serum causes a precipitation of the substance contained in the cell bodies of the diphtheria bacilli, and agglutinates them. Schwone, of Vienna, was able to show that a serum obtained by treatment with a single culture of diphtheria acted only on a limited number of diphtheria cultures; and that, in order to obtain a serum specifically affecting all cultures equally, it was necessary to treat the animal with a large number of different diphtheria cultures, in other words, to make a multipartial serum. In this way it has been possible to obtain sera which, in dilutions of 1 : 5000 or over, specifically agglutinate all

cultures of diphtheria thus far examined. Like Martin, of the Pasteur Institute, I have therefore sought to determine whether any practical application could be made of this serum, which, in contrast to the antitoxic serum, acts on the bacilli themselves.

You are well aware that a great difficulty in combating diphtheria is this: that in healthy children, but especially in diphtheria convalescents, despite the use of antitoxine, the diphteria bacilli often remain in the nasopharynx for a very long time. This is extremely annoying, because a child so affected cannot be sent to school until all diphtheria bacilli have disappeared from the nasopharynx. I have therefore proceeded as follows: A strongly agglutinating, multipartial diphtheria serum is evaporated to dryness *in vacuo*, mixed with sugar of milk, pulverized, and pressed into tablets. These tablets when dissolved in the mouth cause the fluids in the mouth to become stongly agglutinating. The question was, and is, whether this process of agglutination will help us to get rid of the diphtheria bacilli from the nasopharynx more quickly and surely than was heretofore possible. The clinical experiments thus far made speak in favor of the employment of this serum. Whereas it is no rarity for diphtheria bacilli to be present in the throats of convalescents for weeks, it has been found that in the cases in which these tablets have been used the bacilli disappeared within a few days.

My usual method is this: A tablet is allowed to dissolve in the mouth about every two hours, and then, after fifteen minutes, the child's nasopharynx is rinsed out with an indifferent fluid in the form of a spray or gargle. I conceive the action to be such that, whereas when this serum is not employed the diphtheria bacilli are scattered diffusely throughout the nasopharynx, under the influence

of this serum they are agglutinated or clumped together. One might liken the process to that by which we remove dirt scattered about in a room. The dirt is swept together and can then be readily removed. The diphtheria bacilli are massed together more or less by the serum, and these clumps are then removed by the subsequent rinsing. In this way they are so much decreased in amount that the natural power of the organism is able much more quickly to make away with those remaining. I am in hopes that this new diphtheria serum will be destined to be of great service, especially in making prophylaxis easier and in making it possible to send the diphtheria convalescents to school earlier than heretofore.

But not only for serum therapy is my view of the employment of many different kinds of diphtheria cultures of value; I believe that it is equally important for active immunization, *i. e.*, for protection against an infection by means of dead bacteria. In the future such a serum, made by the simultaneous inoculation of various races of one and the same or closely related species of bacteria, will play a more and more important rôle.

You see, gentlemen, that in the subject on which I have spoken this evening, theory is not merely of academic interest, but, on the contrary, plays an eminently practical rôle; and that in the present state of our knowledge we are only then able to further practical science when we carefully regard the theoretical side of this study and, by means of experiment, seek to extend its application.

The principal object of my lecture is to make plain this intimate connection between theory and practice, and this I have attempted to do by means of a few illustrations. In closing, gentlemen, I wish to express my appreciation for the opportunity given me to address this assembly.

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DR. OTTO H. SCHULTZE, *President.*

Dr. F. C. Wood demonstrated stained slides of Trypanosoma hominis and Trypanosoma Brucei. Dr. Wood gave a short discussion of the conditions under which these parasites occur, causing the diseases known as "sleeping sickness" and nagana.

Dr. James Ewing presented Specimens of a Case of Kala-Azar, recently described by Marchand and Ledingham (*Zeit. f. Hygiene*, Bd. XLVII). The sections were prepared from material which Prof. Marchand had very kindly presented to him in Leipsic. The sections showed large numbers of the protozoon described by Donovan, Leishmann, and others, and which had first been found in the aspirated blood of the spleen. While very scanty in the

circulating blood, they were enormously abundant in the viscera. The parasites occupied much enlarged endothelial cells which contained from ten to one hundred in section. The structure of the parasite was very distinct even in the sections stained by haematoxylin and eosin or by Nocht's method. It consisted of a macronucleus and a very densely staining micronucleus inclosed in a protoplasmic envelope from 1 to 2 micra in diameter. It was mentioned that considerable evidence supports the belief that this body represents one phase of the life history of a trypanosome.

THE WIDAL TEST FOR PRACTISING PHYSICIANS.

JOHN H. BORDEN, M.D.

Since the demonstration of the value of Widal's test as an aid in the diagnosis of typhoid fever, various modes of applying it have been in vogue. Dried blood has been used largely for clinical work. For scientific study, the separated serum which permits more accurate dilution, has been employed. Hanging drop preparations observed with a high power of the microscope have been as a rule a part of the technique. Until recently no method which could be applied readily and safely by each physician to his own cases had been suggested, but within the past few years several attempts have been made to introduce a macroscopic Widal test. In each instance the advantages claimed have been:—

1. Ease and simplicity of technique;
2. Independence of the microscope;
3. The use of a permanent suspension of dead bacilli.

In April, 1902, Pröscher (1) described a new Widal technique. He used an emulsion or suspension of 24 hours bouillon-grown bacilli killed with formalin. This he mixed with the diluted serum in little transparent dishes, set them in the incubator at 37° C., and at the end of an hour or two observed the result with a low power of the microscope. The method is hardly adapted to use outside of a laboratory.

In 1903, Ficker (2) of Darmstadt, published a notice of a typhus diagnosticum devised by him and possessing all the advantages of general availability suggested above. He failed to state how he prepared the suspension which was the essential feature of his method, but intimated that it involved a lengthy and complicated process and that it would not be wise for others to try to make a similar solution. Ficker gave no reports of cases in which his diagnosticum had been tried but said it had been used with success, and in a footnote stated that Merck had been entrusted with its manufacture. He suggested that those who wanted any might buy it. The price as put up with sufficient suspension for about twenty-five tests is one dollar and fifty cents.

In August, 1904, Walter (3) reported a series of 22 cases tested with Ficker's diagnosticum. Of these, 15 caused agglutination in high dilution. Of the 15, twelve were undoubted typhoids, 2 were probably typhoid, and 1 which reacted only in a dilution of 1–50, gave no other sign of typhoid fever. The remaining 7 non-typhoid cases did not react. All of the cases were tried at the same time by the usual microscopic method and grossly with a solution which Walter made with agar-grown cultures suspended in a mixture of salt solution, glycerine, and carbolic acid. The results of the three methods coincided.

Ruediger (4), in a recent number of the *Journal of Infectious Diseases*, reports a long series of gross reactions done with formalinized emulsions and states that certain sera diluted 1–20,000 caused visible agglutination in 5 to 8 hours. This is perhaps possible, but after our experience with formalinized cultures it seems likely that owing to insufficient control work he was deceived by albumin precipitations.

Buxton (5) also has met with success while using cultures of bacilli killed with formalin.

Rolly (6) has worked to advantage with a solution in which the organisms are killed with formol or toluol. The preparation of Rolly's emulsion necessitates several weeks time and much care.

Gramann (7) and Kasarinow (8) have reported favorably on Ficker's diagnosticum.

Early last spring, in the Presbyterian Hospital laboratory, we began working with suspensions of typhoid bacilli which had been killed with formalin. With one particular stock of solution we met with marked success, testing a large series of cases of typhoid fever and thoroughly controlling the work with many different non-typhoid sera. All the typhoid cases agglutinated our formalinized emulsion and only two of over 100 different control sera caused apparent clumping within the three hour time limit. One of these cases had certainly had typhoid fever, the other possibly. An approximate dilution of 1–100 was used in each case and if there was no clumping visible to the naked eye at the end of three hours the result was considered negative. The average day of reaction was the seventh to eighth day of the patients sickness in bed. Many of the control tubes showed clumping on standing 6 to 12 hours.

We never made another formalin solution which

abundant control work did not prove unreliable. We attributed this to the instability of the formalin and to the fact, pointed out by Gatrona (9), that this reagent in the presence of a very slight trace of acid constitutes one of our most delicate tests for albumin.

After several further unsuccessful attempts to compound a satisfactory suspension, we made, aided by valuable suggestions from Dr. T. Hastings, a solution in this manner:

A well tried strain of typhoid bacilli is grown for 24 hours on slant agar in large tubes.

The bacilli are washed from the media with a mixture of sterile salt solution, 450 parts, glycerine, 50 parts, 95 per cent. carbolic acid, 1.25 parts. One hundred c.c. of the solution are sufficient to wash one large agar tube.

This solution is at first cloudy, but after standing for two weeks becomes translucent and the bacilli on being transplanted fail to grow. It is then ready for use, and is permanent if kept in sterile bottles in a moderately cool and dark place. The small percentage of carbolic acid, equal to 1–400, renders contamination difficult, and the glycerine by increasing the specific gravity serves to keep the bacilli in suspension. The stock bottle should be shaken occasionally.

With such a suspension and five others prepared in the same way, 30 cases of typhoid fever and 27 controls were tested. Of the 30 cases, 29 agglutinated in an accurate dilution of 1–100. The one case which failed to clump died in the second week after his blood had been twice tested. None of the controls reacted with the serum diluted only 1–40. The controls included 3 cases of nephritis, 2 of malaria, 1 of appendicitis, 2 of tuberculosis, 1 of abscess of the liver, 1 of cholelithiasis—both with jaundice— and several surgical cases. The reaction if

positive occured within 20 hours. Six controls stood for eight days without apparent change of any sort, and many others from 48 to 72 hours. The average day when the reaction was obtained first was the patient's seventh day in bed. Four reacted on the second day in bed, 1 on the third, and 1 on the fourth. Twenty-five cases were positive on the first examination, 2 on the second, and 2 on the third examination. An interval of 48 hours elapsed between successive examinations. The different solutions were uniform in their susceptibility to agglutination except that one made with bacilli grown for 8 days was much slower in reacting than the others. Coincident examinations using Ficker's diagnosticum and the microscope method gave similar results. Ficker's suspension was somewhat slower. At the expiration of a month's use the solution reacted as well as on the first day. Our technique has been the following:– All tubes and pipettes are cleaned before using with alcohol and ether.

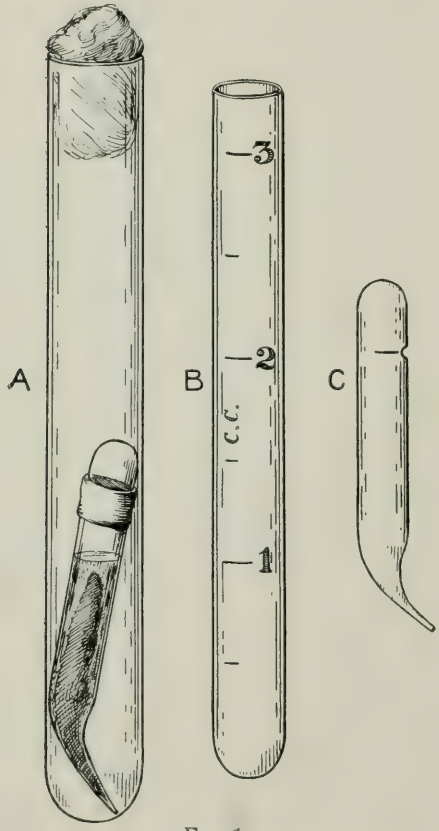

Fig. 1.

A.—Small Test Tube containing the collecting bulb filled with blood.

B.—Graduated Test Tube for rapid dilution.

C.—Collecting bulb showing vent and file mark for breaking end.

1. The blood is collected in small bulbs with a small vent-hole at one end and drawn to a capillary tube at the other. It is only necessary to prick the ear rather briskly with a Hagedorn needle and keep the tip of the bulb to the exuding drop. Milking (not squeezing) the lobe of the ear will facilitate the flow of blood. When the bulb is two-thirds full, it is held for one minute in a slanting manner and laid aside for one to two hours. At the end of this time about 0.2 c.c. of serum will have separated. By covering the vent-hole with a broad elastic band the bulb may be carried any distance. The point may be sealed with sealing wax.

2. From a modified Shuster dropping-bottle a small graduated test tube is filled to the 1 c.c. mark with sterile salt solution. The large end is broken from the bulb at the file mark and with a capillary tube drawn to a long point and graduated in one-hundredths of a c.c., enough serum is drawn up to make with the salt solution the dilution required. The pipette with the contained serum is then inserted into the test tube and thoroughly washed out with the salt solution.

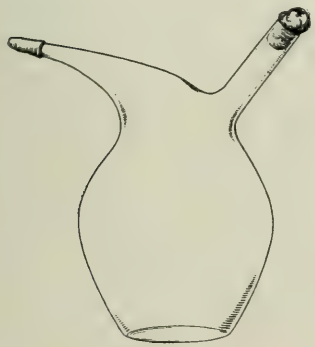

FIG. 2.

Dropping bottle for sterile salt solution.

3. From another dropper enough of the typhoid suspension is added to bring the fluid in the test tube to the 2 c.c. mark. The mouth of the tube is then stopped with a plug of cotton and set aside in a cool, dark place.

Within two to twenty hours, if the reaction is positive, depending upon the dilution of the serum used and its agglutinating power, a marked granularity of the fluid

in the tube will be noted. Following this there will be seen distinct clumps beginning to sink toward the bottom, and at the conclusion of the reaction, the fluid above will be limpid and free from clumps and the point of the test tube will contain a small, white, flocculent, mass of agglutinated bacilli.

In conclusion:—

1. The method seems to be adapted to use by the practising physician because its technique is simple and free from danger, and because the reading of the reaction is absolute. It is either positive or negative.

2. It is possible that a similar suspension of appropriate bacteria may be used in the diagnosis of diseases other than typhoid fever.

3. The composition of the fluid composing the basis of the suspension may perhaps be altered and improved so that the agglutination will occur more quickly.

4. The methods of dilution here suggested are thought to be somewhat simpler and more rapid than those heretofore employed.

FIG. 3. Graduated pipette.

Finally, I wish to thank Dr. T. Hastings for valuable suggestions, and Miss Selma Granat for material aid in preparing the suspensions.

Bibliography.

1. PRÖSCHER, Zur Ausstellung der Widal'sche Reaction. *Centralblatt f. Bakt.* No. 31, 1902.

2. FICKER, Ueber ein Typhus Diagnosticum. *Berl. klin. Woch.* No. 45, 1903.

3. WALTER, Zur Typhus Diagnose. *Deut. med. Woch.* August, 1904.

4. Ruediger, *Journal of Infectious Diseases.* Vol. I, No. 2, 1904, p. 236.

5. Buxton, On Agglutination. *Journal of Medical Research.* July, 1904.

6. Rolly, Zur Diagnose des Typhus abdominalis. *Münch. med. Woch.* No. 45, 1903.

7. Gramann, Zur Serodiagnose des Typhus abdominalis, mittels des Ficker'schen Diagnosticum. *Deut. med. Woch.* No. 22, 1904.

8. Kasarinow, *Russk. Wratch.* No. 53, 1903.

9. Gatrona, Gatrona Test for Albumin. *La Riforma Med.* 1903.

Discussion.

Dr. W. H. Park said that it seemed to him that the method described by Dr. Borden was very much easier for the physician than the old method, but he asked whether there was any difference in results with this method of using cultures killed by carbolic acid, and the usual use, in proper proportions, of live cultures.

Dr. Borden said that he had tried a few live cultures microscopically and they reacted very well. With the method he described the physician did not have to make plants from day to day and keep live bacilli about the house. They reacted, moreover, perfectly well. Widal himself had spoken of using the dead bacilli for his agglutination test.

Dr. F. C. Wood said that he was not willing to give up what had been to him a very important phenomenon—the motility of the bacilli. The bacilli in cases of miliary tuberculosis tested in dilutions of 1–40 and 1–60 showed persistent motility, and it was this motility which differentiated such reactions from true typhoid in which motility ceased early. He asked Dr. Borden if they had had that experience at the Presbyterian Hospital.

Dr. Borden replied that they had.

Dr. Wood asked if they had tried the suspension in cases of miliary tuberculosis.

Dr. Borden said he had tried three cases of miliary tuberculosis by the gross method and did not get the reaction in any of the tubes, 1–40, after standing several hours.

Dr. Philip Hanson Hiss said that he thought Dr. Wood's objection to dead bacilli generally held with the microscopic test. Any action we might get with tuberculosis when using the gross test was not noticeable to the eye, for although we might get very small clumps, he doubted if we would get precipitates.

Dr. E. Libman said that at the Mount Sinai Hospital they had tried the Ruediger method with formalinized cultures. It was found not to be specific. They had had several cases, not typhoid (in two cases of which there was no fever) and the test gave a complete reaction at 1–20 and 1–50. They had given up the Ruediger method. Whether this happened when the serum was used alone, he did not know. With the Ruediger method the blood was diluted. They had obtained microscopic reactions with both live and dead bacilli, and they had found it better to stick to the live bacilli as the results were better. They had had positive reactions with live bacilli when the reactions were absent with dead bacilli. On the other hand, one or two cases were found to give positive reactions with dead bacilli when not positive with live. He said that microscopically, when we do get a good reaction with dead bacilli, it is as a rule better and more decided than we get in the same case with the live bacilli.

Dr. Borden said that the apparent success which he at first had with the bacilli killed with formalin was very

deceiving. For a long time the reaction was thought to be specific; it did not always occur in three hours, but in the course of five or six hours a flocculent precipitate would be seen coming down. Rather arbitrarily a standard of three hours was set. This was thought to be all right, but the solutions as made up may have been a little too acid and the reaction commenced to occur before the three hour limit. Finally, after trying a great many emulsions, the method was decided to be worthless. Whether other observers use a different method in making the suspension, Dr. Borden did not know, but apparently they used about the same. He had gained the impression in reading the articles that they had not done sufficient control work to make their results convincing. He himself had done more controls than cases.

PATHOLOGICAL STUDIES IN RABIES.

D. W. POOR, M.D.

The histological study of rabies material was undertaken a few months ago at the Health Department Laboratory, at the suggestion of Dr. Park, who wished to have some of the newer methods applied to it, with the idea of finding some diagnostic condition, or, if possible, the cause of the disease itself. The work done so far is in no sense complete. The idea in bringing it to the attention of this Society is to obtain from those who may be interested in this subject, their ideas as to the nature and significance of certain conditions found, and suggestions as to future lines of work.

The most striking and constant condition found thus far is the presence of certain minute bodies, described in

1903 by Negri, a worker in Golgi's laboratory, and considered by him to be protozoa and the cause of the disease.

We have used material in this study from sixteen dogs, nine guinea-pigs, three rabbits, and from one case of human rabies. Of the dogs, five were ordinary cases of rabies, as they came from the street. In five the disease was produced experimentally, twice by subdural inoculations of fixed virus, once by subdural inoculations with virus from the submaxillary gland in a dog which died of street rabies, and twice by inoculation into the leg, in one case the virus being contained in the gland tissue, in the other in the brain of dogs which had succumbed to street rabies. Six dogs were normal and were used as controls.

Of the rabbits, two were inoculated subdurally with fixed virus and one was a control.

Five of the guinea-pigs were inoculated subdurally with the brain tissue of dogs which had died of street rabies; one was inoculated with fixed virus; two were normal, and one a mild case of tetanus.

By varying the conditions as to strength of virus, site of inoculation, and variety of animal used, the clinical aspect of the disease was varied in its duration of incubation and of symptoms

There were in all, then, nineteen cases of rabies and nine controls, of which eight were normal animals and one a case of tetanus.

The first three specimens were fixed in formalin and badly handled so that they were unfit for study. Barring these, there were sixteen cases of rabies examined, in all of which bodies such as have been described by Negri were found. In none of the normal cases was anything

similar seen. In the cerebellum of the case of tetanus, a very few minute inclusions were found resembling granules found in large numbers in animals dying with fixed virus.

In attempting to describe these corpuscles, as they have been called, one finds such great variations in their size, shape, and structure, that the question naturally arises as to whether they are all one and the same thing in different forms, or are sometimes of different nature.

Shape.—The commonest shape seen is round, next oval or oblong. Occasionally irregular shapes are seen which might almost suggest a previous amœboid motion.

Size.—The size also varies, the largest forms being half again as large as a red blood cell, or even larger, while the smallest appear as minute granules scarcely visible with a 1–12 oil immersion lens.

Structure.—With regard to structure we find again the greatest difference. Some of the largest bodies appear to have a certain definite structure. For example, they may contain from one to a dozen or more small round granules, each surrounded by a circular clear space. These granules are sometimes arranged in a circle around an irregularly shaped center. Sometimes they fill the corpuscle completely. In other instances, we find little structure, except that the periphery stains more heavily and has a notched appearance, in places giving the appearance of ring bodies. Other corpuscles stain solidly throughout and are without apparent structure. The smallest forms appear as minute granules. This difference in structure may be due in part to variation in technique.

Numbers.—In some of the brains examined, the corpuscles were very numerous, appearing as inclusions, sometimes several in a cell, throughout the cerebrum and

cerebellum. In other cases, they were few in number, being seen only in a limited number of the Purkinje cells of the cerebellum. When very numerous they may appear outside of the ganglion cells. The Purkinje cells and the large ganglion cells in the region of Ammon's Horn are given by Negri as the most favorable regions for finding them. Some of the smallest forms, or bodies like them, occasionally appear within the nuclei.

According to Negri, when the animal has been inoculated subdurally they are rarely found in the cord and then only in the cervical region, but when the inoculation has been into the sciatic nerve, they are much more numerous in the cord, especially in the lumbar regions. We have examined but one cord and are therefore unable to give any personal experience on this point. Although in our cases the greatest number of corpuscles appeared in a guinea-pig inoculated subdurally and the fewest number in a dog with rabies contracted on the street, yet they were found in large numbers in other dogs with street rabies and in a pup which was inoculated in the leg, so that the site of inoculation does not always seem to affect the numbers present in the brain.

Although Negri claims to find all fixations equally good, it has seemed to us that Zenker's solution is by far the best for this work. Alcohol and formalin with their various combinations are very uncertain. Bichloride of mercury answers well.

In the main, all these different forms agree in being acido-phile in their staining properties. The best stain is an eosin-methylene blue combination, with which they present a striking appearance as red inclusions in the blue body of the nerve cell. They may also be stained with the haematoxylin and eosin combination, with Borel's stain, iron alum haematoxylin and fuchsin.

They failed to stain with thionin in the only specimen tried. With the eosin-methylene blue stain, some of the corpuscles, especially the larger structured forms, take on a purplish or almost bluish color; others appear to be a light green. Others show as a red body with blue nuclei. In the main, however, they stain bright red.

Nature.—The nature of these corpuscles is a matter of speculation. Negri asserts that they are protozoa. In support of this view are the apparently highly structured, purplish bodies occasionally seen, which present a rather startling appearance. That they are not artefacts is clear from the fact that they do not appear in normal tissue treated in exactly the same way.

The suggestion has been made by Dr. Williams, of the Health Department, who has examined all the preparations and given much valuable advice, that they may be degenerating red blood cells. As supporting this idea are the facts that the brain tissue is markedly congested in rabies, that red blood cells appear in close proximity to the ganglion cells and further than undoubted red cells do occur as inclusions in the ganglion cells. The staining properties of the corpuscles and of the red cells are practically identical. Further, in many instances the red blood cells have considerable structure, presenting somewhat suggestive intermediate forms. However, it must be said that red blood cells with as great an amount of structure as the most elaborate of the corpuscles are not seen, at least not within the blood vessels.

The fact that a few minute eosinophile granules were found within the Purkinje cells in the one case of tetanus examined is suggestive that these may be degeneration forms of some structure, possibly the intranuclear network. After all, these small granules, the only forms

found in animals poisoned by fixed virus, may be of an entirely different nature from the large structured forms which Negri insists are only found when the incubation is prolonged to two weeks by using street virus.

I have not been successful in obtaining the haemosiderin reaction in these corpuscles in the two trials made.

Another point to be considered in discussing the nature of these forms, is the fact that by using the same fixations and stains they do not appear in the submaxillary glands examined thus far. These glands are infectious and while they show certain bodies of necrotic nature, Negri corpuscles as they appear in the brain have not been seen by us.

Whatever the nature of these inclusions it would seem as though they might be of considerable diagnostic importance. We have as yet examined practically no pathological cases as controls. The findings in the case of tetanus examined showed none of the large forms characteristic of street rabies, which is the form of the disease requiring diagnosis practically. It is hoped that those who have examined the brain tissues under various pathological conditions will give us their experience on this point. The presence or absence of these forms in other brain affections is of course a most important point in determining their diagnostic value, but it would seem from our experience that the material should be fixed in Zenker's fluid to make the control accurate.

Negri has subjected this method of diagnosis to a rather severe test having diagnosed rabies in 70 out of 71 cases correctly. Further, he did not diagnosis it in any case proved not to be rabies, He ascribed his failure in the one case to the fact that he had a very small portion of tissue from only one portion of the brain.

I may add in conclusion that in our very limited experience, in 3 out of the 4 cases of street rabies in dogs, the diagnosis was very easy, the inclusions being very numerous. In the fourth they were few, their discovery requiring the searching of many fields. It would seem that for purposes of diagnosis tissue should be taken both from the regions of Ammon's horn, the cerebellum, and if possible from the lumbar cord.

Discussion.

Dr. Anna Williams said that on first studying the specimens the possibility of the cell inclusions being derived from the red blood cells seemed great, but the further the study was carried, the less the possibility seemed. The majority of the forms she had seen showed such a constant, definite and characteristic structure, that it seemed almost impossible to consider them derivatives of red blood cells. Furthermore, a few smears which she had made and stained by the Nocht-Romanowsky method, as recommended by Dr. Ewing, stained the bodies more or less characteristically and showed no evidence of their having been derived from red blood cells or of their being cell degenerations of any kind. Dr. Williams said therefore, that as far as morphological study of stained specimens alone, could help, the evidence was in favor of the inclusions being individual organisms or protozoa.

Dr. James Ewing said that he had the pleasure of looking over some of Dr. Poor's specimens and that he had drawn impressions regarding the nature of the Negri bodies in the reverse order of those expressed by Dr. Williams. In the first cases examined, the very definite structure of the bodies in the ganglion cells, and the fact that they were totally unlike anything he had ever before

seen in a considerable experience with ganglion cells examined under a high power of the microscope led him to think that they must be foreign bodies and probably protozoa. After seeing the specimen from the guinea-pig, in which the disease was of slow development, he had found, on the contrary, much evidence that the red cell theory suggested by Dr. Williams, was well founded, and would be a safe one to follow as a working hypothesis. It was well known that the cyanotic blue color of the cortex in rabies was pathognomonic and was referable to extreme distension of the blood capillaries of the gray matter. In the case of this guinea-pig, not only were the capillaries distended, but there was considerable diffuse infiltration of the gray matter with whole and fragmented but otherwise unaltered red cells. Many of these cells lay in the lymph spaces about the ganglion cells, and in the bodies of some ganglion cells there were found unaltered red cells. In this guinea-pig, but not in the other cases he had examined, there appeared to be numerous transition forms between red cells and the parasites. The argument from transition forms, however, Dr. Ewing did not regard as free from danger of error, and he had never seen any such degeneration forms of red cells in any other condition. Experience had shown that whatever their nature these bodies were diagnostic of rabies. The protozoon theory, Dr. Ewing thought, ought not to be hastily welcomed until other less sensational hypotheses had been fully set aside by systematic control work. He had suggested that the staining of cells isolated by teasing and dried on a slide might give some information about the bodies more reliable than that obtained by the study of sections of tissue fixed in complex mixtures of metallic salts and acids. The fact that the bodies were not found in the parotid and submaxillary glands, and

that the virus of rabies passed the Berkefeld filter, seemed incompatible with the view that the Negri bodies were the protozoon cause of rabies.

SOME OBSERVATIONS ON THE PATHOLOGICAL CHEMISTRY OF TUMORS.

S. P. BEEBE, M.D.

Dr. Beebe presented the results of studies on the pathological chemistry of tumors which he thought would be particularly interesting to the physiologist and biologist.

The first study of tumors was made upon fresh material to determine the condition that exists in the tumor during its period of growth. The tumors which were used for this study were all removed at operations with one exception. This was a post-mortem case. The striking thing about this tissue as compared with normal tissue was that there seemed to be large quantities of autolytic material stored up, which is not strange as the tissue is rich in cells and the circulation is very poor. In a case of carcinoma of the broad ligament, Dr. Beebe had found a substance which has been found only once before free in the tissue—glycocoll or amido-acetic acid—a very soluble substance, which undoubtedly exists floating about the body in considerable quantity, because the addition of benzoic acid results in a synthesis with itself of large quantities of hippuric acid. This substance was present in considerable quantities. Dr. Beebe did not know how to account for it except by autolysis; the tissue was sterile. The tumor was growing upon a tissue which

might be expected to give glycocoll—the broad ligament —a tissue yielding large quantities of collagen and consequently of glycocoll. The tumor tissue was in this respect like the tissue upon which it was growing. The poor circulation would not carry this substance away. Other amido acids, leucin, tyrosin, tryptophan, were found in abundance, not only in tissues decomposed but also in one sarcoma which was rapidly growing and without any degeneration as far as could be seen. Another substance found was a peculiar kind of carbohydrate, resembling in some ways starch, and in some ways, glycogen, a substance which gave a color reaction with iodine like starch, and was readily dissolved in water. By hydrolyzing this solution with dilute hydrochloric acid, the color reaction went through the changes from blue to pink and wine red, like the reaction of glycogen. On more complete hydrolysis there was a reduction of Fehling's solution which showed that some kind of reducing carbohydrate was present which was not glycogen nor starch, but something between the two.

All of these tumors showed products of autolysis. One showed large quantities of products which are commonly found in autolyzed tissues. It seemed to be an admirable place for autolysis to go on. The circulation is impaired so that the degeneration of the tissue may take place; the temperature is favorable; and the tissues are sterile.

Some recent work was more interesting. This had been a study of the inorganic salts, nitrogen, phosphorus, sulphur, potassium, and calcium as found in the ash.

The tumor tissue was thoroughly pulverized with the hashing machine, dried in the oven, first at a low temperature, 60°, which was gradually raised until the final drying was accomplished at 108° to 110°.

These results show nothing of much interest for the sulphur, phosphorus and nitrogen, nor are they remarkable as to potassium and calcium, but they are to some degree interesting. The findings for these salts are given in two different sarcomas, as follows:—

	Calcium	Potassium
Sarcoma 17	1.144	0.060
Sarcoma 18	0.900	1.625

These two were of the same type but in different conditions. Sarcoma 17 was very badly degenerated; sarcoma 18 was a very fresh tissue. It was rapidly growing, a round-celled sarcoma. The point of interest was that No. 18 was fresh tissue, rapidly growing, and contained a large percentage of potassium. In the dried tissue, 100 grams of tumor contained 1.625 potassium.

There was a comparatively small quantity of calcium and in the degenerated tumor the conditions were just reversed; there was a large quantity of calcium and a comparatively small quantity of potassium. This change may be a progressive one. In a carcinoma of the liver, kindly furnished by Dr. Schultze, primary in the pancreas with metastases in the liver, Dr. Beebe was fortunate enough to get a portion of the primary tumor, secondary tissue cells, and some of the normal liver tissue. The result were as follows:

	Calcium	Potassium
Primary pancreas	0.1420	0.3831
Secondary	0.1150	0.9740
Liver	0.0700	0.8265

The primary growth in the pancreas was somewhat degenerated and the results show that there is less potassium and more calcium in this tissue than in the secondary growth in the liver.

There may be even a progressive change in the organic cell content with degeneration of the tumor tissue.

These results are of interest because of the apparent antagonism of calcium and potassium in their physiological action. One of the striking pieces of work showing this was done last winter by McCallum at the University of California. A small quantity of potassium chloride was injected into one of the arteries of the mesentery with a resulting increase in the secretion of intestinal glands. An active peristalsis was set up in that part of the intestine supplied by the artery. An application of the salt was made to the peritoneal surface of the intestine with the same result; this affect was inhibited almost instantly by the use of calcium.

In rapidly growing plant tissue there is a much larger quantity of potassium than is to be found in the older plant tissue.

Another instance pointing to the importance of these elements in an active tissue is the finding obtained by the analysis of milk. Only a few analyses have been made extending through lactation, and these have been made on the lower animals. In the beginning of lactation in one case, 20 per cent. of the milk ash was potassium. This quantity increased until the second month, the time of the greatest milk secretion, when the quantity of potassium in the milk ash was 29.5 per cent. Then it fell slowly. In the last two months it fell quickly until the last analysis made showed 15.26 per cent. potassium.

The calcium either does not change at all or increases slightly during the course of lactation. In one case there was no change, no decrease nor increase of the calcium from the beginning of lactation to the end. These salts are not present in milk as simple filtration products from the blood. In fact, it is customary to

suppose that the mammary gland is so active that the cell substance itself is destroyed by this activity and the author suggested that these inorganic salts present in the milk represent the condition in the secreting cell and are a measure of the salt content in that cell. If that be so, and it is a point which Dr. Beebe expects to study during the winter, we have another instance of a very active cell and tissue containing a much larger quantity of potassium and a smaller quantity of calcium than resting tissue.

The growth of a tumor is a series of active nutritive changes. In those tumors that have the most active nutritive changes, the speaker had found this large quantity of potassium and in those that are comparatively inactive, where only autolytic changes were going on—those of destruction and break-down—a large quantity of calcium was present and a small quantity of potassium.

Dr. Beebe did not wish it to be understood that this was in any way a suggestion leading to a new theory as to the cause of cancer. He had made analyses for inorganic salts in only nine tumors, which is a small number, but the results found in those tumors seemed to him to harmonize with what is found in other cases, and emphasized the importance of the physico-chemical environment of the cell.

Discussion.

Dr. F. C. Wood said that Dr. Beebe's findings of glycocoll in the products of autolysis of the tumor cells was especially interesting to him as he had been recently attempting to isolate glycocoll from the urine of a patient with acute yellow atrophy. Leucin and tyrosin had long been known as products of liver autolysis in acute yellow atrophy. Recently Fischer had devised a new method

for isolating the amino acids and glycocoll had been found in the urine in experimental phosphorus poisoning in dogs, but as yet he knew of no records of the finding of glycocoll in acute yellow atrophy. It would be natural to expect, however, that this member of the amino acid group would be present in the urine.

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Dr. Otto H. Schultze, *President.*

BILE DUCT ADENOMATA OF THE LIVER.

Eli Moschcowitz, M.D.

Dr. Eli Moschcowitz presented a case of bile duct adenoma of the liver. The liver was removed from a patient who died at the Roosevelt Hospital on April 27, 1904. No history of the case was obtainable, but the clinical diagnosis was cerebro-spinal meningitis. The autopsy was performed by Dr. Ditman. In addition to the characteristic lesions of cerebro-spinal meningitis, moderate atheroma of the valves of the heart and a healing tuberculosis of the right apex were present. The liver was larger than normal and yellowish brown in color. It was uniformly studded with small nodules, gelatinous in appearance and varying in size from the head of a pin to that of a split pea. They were very soft, sharply de-

fined, with no definite encapsulation, and fine prolongations could be seen passing into the liver substance. The organ otherwise showed slight fatty degeneration. Microscopical examination showed an adenomatous structure, the alveoli being round, oval, or tortuous in character. They were lined with low cuboidal epithelium with a large round nuclei, in various stages of degeneration, particularly on those alveoli situated in the central part of the nodule. The lumina of the alveoli were filled with a finely granular or hyaline mass, staining evenly with eosin; but in a few instances were filled with masses of desquamating epithelium. The alveoli were enmeshed by dense masses of connective tissue which was fibrillar and non-cellular in the central part but very cellular toward the periphery. The cellular structures of the latter consisted of round cells and many newly formed bile ducts as are ordinarily seen in cirrhosis. These bile ducts in many places could be traced from the various stages of simple canals with no lumen to the cystic changes already described. There was no capsule, the nodules lying directly against the liver substance. There were but few blood vessels A marked characteristic of these nodules was their intimate relation to Glisson's capsule. The normal bile ducts of the liver showed no changes. In occasional sections the lumen of the portal vein was found to contain large cells resembling in many respects liver cells. There was very little diffuse cirrhosis and the liver cells showed no changes except a moderate fatty degeneration. The picture as a whole was that of an adenomatous hyperplasia of the biliary ducts.

The cases in the literature of this character were very few and resembled the one reported in almost every particular, except in the size of the new formations. The livers as usually described were riddled with cystic for-

mations varying in size from a cherry to a child's head. So far as Dr. Moschcowitz could ascertain his case was unique in the number and the uniformly small size of the tumors. The interesting point in connection with the case was their pathogenesis;—as to whether the tumors were inflammatory in character or were new growths. Dr. Moschcowitz had at first inclined to regard them as new growths, but after fuller observation had come to the belief that they were an evidence of inflammatory hyperplasia. While it could not be definitely proven that they were not new growths in the strict sense of the term, nevertheless there were many features present that strongly suggested an inflammatory origin. While there was no very extensive cirrhosis present, there was a distinct proliferation of new connective tissue in Glisson's capsule with the formation of new bile ducts such as we often see in cirrhosis. Furthermore, one could easily trace the different stages, from that of the newly formed bile ducts to that of the mature and well formed alveoli. A further evidence of a progressive inflammation lay in the slow transition from the cellular fibrous tissue in the periphery to the advanced and fibrillar connective tissue in the center. The whole picture represented a centripetal process rather than a centrifugal process such as we would expect in a new growth formation. In certain areas, old and degenerated liver cells could be found in the central part of the nodules. This might also be construed as an evidence of old inflammatory changes similar to those found in cirrhosis. There was no evidence of encapsulation as we might expect in non-malignant new growths and as is invariably found in all other adenomata of the liver. An additional evidence that the lesion was an inflammatory one lay in analogy:—there was a great similarity in the microscopical picture to that of other in-

flammatory tumors of the liver, namely, coccidiosis. The majority of observers have regarded these nodules as new growths, but as only the advanced stages have been described this may account for their being so regarded. In an early specimen the inflammatory changes would perhaps be far more apparent then in the advanced stages in which this condition is usually seen. The cause of the dilatation of the newly formed bile ducts was hard to trace. Unfortunately, the history was not obtainable in this case, wherein there might have been found some evidence of an old inflammatory process. A distinct history of jaundice had been observed in a few of the cases described. Dr. Moschcowitz thought there was a possibility of there having been a cholangitis or some other form of obstruction of the biliary passages.

PHOTOMICROGRAPHIC APPARATUS USING ULTRA VIOLET LIGHT.

S. CZAPSKI.

Prof. S. Czapski demonstrated the new apparatus for the use of ultra violet light for photomicrographic purposes as manufactured by Carl Zeiss of Jena. Prof. Czapski first discussed the methods which have been used up to the present time to increase the efficiency of the microscopic objective. He showed that the resolution was dependent upon the aperture, and upon the wave length of the light used in the objective. The ordinary objective uses a light of a wave length of about five hundred and fifty millionths of a millimeter. Attempts which had been made to increase the resolving power of the

objective by the use of glass of a higher refractive index combined with an immersion fluid of high-refracting power had resulted in the production of an objective of an aperture of 1.60, using monobrom-naphthalin as the immersion fluid. The difficulty of obtaining mounting fluids of this high refracting index has greatly limited the use of such objectives, so that at the present time this means of increasing the aperture of the objective may be considered as having reached its limit. The other method of improving the resolving power of the objective is to use light of a shorter wave length. It has been found possible by the use of quartz lenses, quartz condensors, and quartz slides and cover glasses to obtain an objective the aperture of which by ordinary light is 2.5, through the use of monochromatic light of the ultra violet region, whose wave length is two hundred and seventy-five millionths of a millimeter. The resolving power of these objectives which possess a numerical aperture of 1.25 is practically twice that of the ordinary apochromatic immersion lens.

The ultra violet rays are obtained by an electric spark passing between cadmium electrodes. The spark spectrum of cadmium gives a series of strong lines of a wave length of two hundred seventy-five millionths of a millimeter. The light derived from the spark passes through two quartz prisms in order to select this particular group of rays, and is then brought to a focus by a condensor, also of quartz. The light then passes through the object to be photographed, is collected by the objective of the microscope and the image thrown on a photographic plate by means of a quartz eyepiece.

Inasmuch as rays of this short wave length are not visible to the eye, no light, in the ordinary sense, passes through the microscope. The rays can be rendered visi-

ble only by the use of a fluorescent screen inserted in the eyepiece. When the image so formed is observed a picture can be seen the details of which are very imperfect. An approximate focus is thus obtained, the camera is swung into place, and the photograph taken. If the focus be correct no further procedures are necessary. If, however, the picture does not appear sharp the focus is slightly changed and a second exposure made. If this is not so good as the first the focus is changed slightly in the other direction, etc.

The mounting fluids which can be used are those which are transparent to ultra violet light;—For example, water, glycerine, and a saturated solution of chloral hydrate in glycerine.

Prof. Czapski demonstrated a number of photographs taken by means of this apparatus, which showed very remarkable results. Especially striking were photographs of yeast cells, which showed structures not visible under ordinary conditions; a photograph of a trans-section of a frog's eye, showing that the lens, while transparent to ordinary light, is opaque to ultra violet light; and also some photographs of Pleurosigma angulatum at a magnification of twenty-five hundred diameters, which showed structures entirely different from those obtained with ordinary objectives.

STRUCTURE OF VACCINE BODIES IN ISOLATED CELLS.

JAMES EWING, M.D.

After many unsuccessful attempts to secure satisfactory specimens of vaccine bodies in isolated cells, a procedure which may be called the "Klatsch method" proved

entirely successful. A glass slide washed in soap and water and heated in a Bunsen flame is found to be unusually cohesive. Such a slide is lightly applied to a cornea presenting any stage of the vaccine lesion, and quickly withdrawn without pressure or lateral motion. It will be found to have carried with it an impression of the lesion in the form of isolated cells which have been loosened by the œdema of the inflammatory process. Repeated impressions will completely excavate the ulcer. The cells dry instantly in the air and may be fixed and stained as are blood smears. The best results were obtained from fixation in 95% alcohol, 10 min., and staining by Nocht's method, 10 min. By this method the epithelial cells and the vaccine bodies are found under much the same conditions as are malarial plasmodia in blood specimens, and their structure and relations are very much clearer than in sections of hardened tissues. This method has been applied to the vaccine lesions in rabbits and rats. Rats appear to be more susceptible to vaccinia than are rabbits, and the vaccine bodies in rats are usually larger than in rabbits.

In "Klatsch" preparations stained by Nocht's method, the first trace of the vaccine body appears in a thickening and dark red stain of several adjoining meshes of the cyto-reticulum. This area is usually found near the nucleus but in superficial cells and in some of the deeper ones, it may lie some distance from the nucleus. By gradual extension of this reticular structure, almost the whole of the cytoplasm may be transformed into vaccine body, while the nucleus, without showing signs of compression, lies to one side of the cell. During these developmental stages, the reticulum of the vaccine body appears to be directly continuous with the cyto-reticulum. In rabbits the cyto-reticulum of the epithelial cell usually

appears much finer than in the rat, and a similar difference appears in the reticulum of vaccine bodies in these two animals. After some accidents of fixation, the cytoplasm of the cell may appear granular, in which case the vaccine body has a granular appearance. The majority of vaccine bodies in isolated cells show definite connections with the nucleus, and in many cases it is difficult to determine where the vaccine body begins and the nucleus ends. The staining reactions of the vaccine body also indicate that it contains chromatin. The meshes of the reticulum of the vaccine body have the usual appearance of cyto-reticulum. They may appear to contain a central granule but this granule always shows connections with the adjoining reticulum and resolves itself into a nodal point of the underlying reticulum. The meshes usually contain homogeneous basophile material, and sometimes the meshes are widened into large spaces containing the same material. Several staining methods were employed in the endeavor to demonstrate some definite structure in the meshes of the reticulum but without success.

After full development of the reticular structure, two series of changes appear in the vaccine body. In some the reticulum in part breaks into granules. This change usually affects the periphery of the body, but sometimes irregular granules appear at different points within the body. These granules are parts of the reticulum and are not derived from any material that lies within the meshes of the reticulum. Similar granules some times spring from the cytoplasm outside of the vaccine body. The second series of changes consists in the appearance of basic staining material in the center of the vaccine body, either diffusely or in one large space, and the gradual increase of this material at the expense of the red reticulum until the entire body is transformed

into a homogeneous basic staining mass which has the appearance of mucus.

That the bodies described are identical with the vaccine bodies in sections is shown by several considerations. The bodies described appear constantly after vaccination of the cornea of rats and rabbits, but are not seen in normal cells nor after inoculation with various bacteria and bacterial toxines. There are no other bodies in the epithelial cells which can be regarded as genuine vaccine bodies if the ones described are spurious. The general type of structure of bodies in isolated cells and sections is identical. The more compact appearance of vaccine bodies in sections may be reproduced in isolated cells, by staining these cells after imperfect fixation which allows the vaccine body to shrink.

In isolated cells stages of the englobment and destruction of the epithelial cell by another are readily followed. The terminal stages of this process yield appearances which resemble some forms of cell inclusions seen in sections, especially those with homogeneous basic centers and large irregular fuchsinophile granules. Englobed leucocytes present very characteristic appearances.

Besides the vaccine bodies other forms of perinuclear cytoplasmic degeneration appear in the epithelial cells. The study of isolated cells failed to show that the full series of vaccine bodies can be reproduced by inoculation of the cornea with various bacteria and bacterial toxines. Diphtheria toxin produces small vaccine-like bodies in superficial cells and homogeneous perinuclear masses resembling the last stages of degeneration of the vaccine body.

A reopening of the discussion regarding the nature of vaccine bodies would seem to be required in view of the new information supplied by the study of these struc-

tures in isolated cells. In "Klatsch" preparations the clear zone separating the vaccine body in sections is found to be an artefact, and the reticulum of the developing vaccine body is found to be continuous with the cyto-reticulum. The apparent continuity of the reticulum of the vaccine body with the cyto-reticulum demonstrates that the vaccine body is a portion of the cyto-reticulum. In the meshes of the vaccine body, no definite structure could be demonstrated. The altered staining reactions of the cyto-reticulum and the close connections of the vaccine body with the nucleus indicate that chromatin from the nucleus diffuses along the cyto-reticulum and enters into the composition of the vaccine body. The chromatin of the large vaccine body may be in part, and of peripheral vaccine bodies in superficial cells may be entirely, derived from the chromatic element which, according to R. Hertwig, is mingled with achromatic substance in the cytoplasm of the cell. Amœboid motion seems to be quite impossible in a structure showing the relations of vaccine bodies in isolated cells. The terminal stages of the vaccine body show that it usually degenerates into mucus-like material. The granules sometimes seen in vaccine bodies are structureless portions of the reticulum.

In the superficial cells of the cornea, there are often very large numbers of granular or ring-shaped structures from 1 to 2 micra and smaller in diameter, the nature of which it is difficult to determine. The larger ones look like the early ring-shaped malarial parasite, and possess chromatin granules, or they may be elongated into rods like bacteria. The smaller members of the series are indistinguishable from cell granules which cover the superficial cells. In these cells congeries of these rings seem to be massed into small vaccine bodies. Similar rings

and granules are found in normal rats and rabbits. In isolated cells the structure of the vaccine body is very similar to that of the chromidial network described by R. Hertwig and others in the cytoplasm of protozoa. In "Klatsch" preparations, the resemblance of the vaccine body to the chromidial network depicted by Zuelzer, *Arch. für Protistenkunde*, Bd. IV, p. 240, is so striking as to suggest that the formation of the vaccine body is in some degree a parallel phenomenon with the development of chromidial substance in the protozoan cell. The relation of the vaccine body to the nucleus also favors this point of view. The parallel, however suggestive, is far from complete, but the observations on chromidial substance demonstrate that nuclear proteids may readily pass into the cytoplasm in the protozoan cell, and Albrecht's study of the nuclear membrane shows the possibility of the same process occurring in the metazoan cell. Yet nowhere else has the extrusion of chromatin into the cytoplasm been found to take the form of the vaccine body.

Although the structure of vaccine bodies in "Katsch" preparations is lacking in any definite feature of a protozoon and offers strong evidence against the protozoon theory, there remains several hypotheses on which it may still be claimed that this body harbors the organized virus of vaccinia. It may be supposed that while the visible vaccine body is a cytoplasmic structure, yet it contains a submicroscopic organism. Or it may be supposed that the vaccine body represents a mixture of the protoplasm of the host cell and the parasitic organism, forming a mycoplasm, such as is believed to exist in Wheat-rust by Eriksson and Tischler, in a disease of grape-vines known as La Brunissere, by Debray, and in crown-gall, by Toumey. But the mycoplasmic theory has not been de-

monstrated even for plants, and the late stages of vaccine bodies show less and not more structure than the early stages. Or it is possible that some other method of fixation and staining than the one employed in this study may yet succeed in demonstrating some definite protozoon structure or character in the vaccine body.

Since more or less specific forms of cell degeneration occur in various infectious processes, it does not seem likely that the vaccine body can be reproduced by another agent than vaccine, and accumulating evidence goes to show that this body is specific for vaccinia and the diseases closely related to it.

SPECIMENS OF CEREBRAL LESIONS.

CHARLES NORRIS, M.D.

Dr. Charles Norris presented a series of specimens showing the effect of cerebral lesions. The first specimen was a brain from a woman who died at Bellevue Hospital. Four days before admission to the hospital she had had an attack of partial unconsciousness. The muscles had become rigid and she had fallen to the floor. When picked up she was found to be paralyzed on the right side of the face and in the right arm. She also had motor aphasia. The leg was uninvolved. In the course of several days she was able to walk around and slowly recovered motion in her right arm, but never in her face. She never recovered the ability to talk, though she regained almost complete use of her arm. Three weeks after the first attack she had another attack, from which she did not recover. The right side of the face was partly paralyzed. She passed without warning into a semi-stuporous condi-

tion. The heart action was very slow and slightly irregular. She died about six weeks after the first attack. At autopsy the heart showed chronic mitral endocarditis with stenosis and recent vegetation. There was general congestion of all the viscera. On opening the skull there was complete cystic softening of the precentral convolution, which had practically disappeared except in the upper part, where the leg center is situated. The postcentral and the whole of the third frontal convolutions were likewise softened. The area of softening involved the first temporal and extended into the supramarginal, but not the angular gyrus. Here the softening was less marked. After the second attack in the hospital the patient had been unable at any time to read. She apparently did not understand what was said to her. She had sensory aphasia. It was hard to understand why she had recovered motion in her arm so quickly after the first attack when there was total softening of the arm center in the central convolution.

The second specimen was a carcinoma of the brain following carcinoma of the breast. Very little history had been obtained in this case. The patient had been operated upon elsewhere for the tumor of the breast and successfully, but she had re-entered the hospital for a recurrence. She was thought to be insane and was transferred to the psycopathic ward at Bellevue. She was in a very confused state of mind and had a paralysis of the right side. At autopsy the growth was found to involve the motor centers of the arm and leg.

The third specimen was from a case of cerebral embolism. The patient was a man who had been six weeks in the hospital. He was apparently in a very stupid condition during most of the time, and after four weeks suddenly became comatose. He had hemiplegia. At autopsy

the brain showed an area of cystic softening involving the Island of Reil, the claustrum, the external capsule, and the lenticular nucleus. The origin of the embolism was undoubtedly in the aorta, this being the seat of marked thromboarteritis. The valves of the heart were fairly normal.

A CASE OF DERMATOBIA NOXALIS, WITH DEMONSTRATION OF LARVA.

HARLOW BROOKS, M.D.

Dr. Harlow Brooks presented specimens from a case of Dermatobia noxalis, the cases of human infection being so rare as to warrant his calling attention to this instance. He was indebted to Dr. Gillette for the specimens. The patient had been for some time in Panama, where his general health had been good until about six weeks ago, when he first noticed three small lumps, one in the groin, one on the flexor surface of the forearm and the third over the left shoulder. The patient declined surgical attendance on account of insufficient hospital facilities and returned to this country for treatment. Examination showed at the apex of each tumor a small perforation through which a small, moving, black body could be made out. The pain caused by the swellings was considerable, and there was a slight rise in temperature. The tumors were opened under cocaine and the worms squeezed out. The largest larva was two cm. long and nine mm. in breadth. There was a considerable collection of pus in one of the tumors, but all of the wounds healed rapidly. Examination of the blood showed a quite marked increase in the eosinophiles. Dr. Brooks said

that the larvæ had been frequently seen in the skin of various animals. He had seen it in the skin of the semi-domesticated buffalo and in the skin of wild prairie-dogs and wild deer. There were some sixty-four different varieties of the bot-fly and consequently sixty-four varieties of the larva, and he could not say to just what variety this specimen belonged. Thirteen varieties had been found to infect the skin, and many of these had been seen in man. Dr. Brooks showed also a parasite from the skin of the buffalo which differed from the human parasite in the arrangement of the spines. He said that he had been told by travelers in South America that the infection there in man was fairly common.

Discussion.

Dr. James Ewing said that he was very glad to see the specimen presented by Dr. Brooks and especially to learn the name of the parasite, as it cleared up the identity of a similar parasite which had been sent to the Cornell Laboratory by Dr. Alexander Lambert. This specimen was identical in appearance with the one shown by Dr. Brooks, and was removed from a patient who gave much the same history and had recently come from Panama.

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Dr. Otto H. Schultze, *President.*

SARCOMA OF THE SMALL INTESTINE WITH EXTENSIVE METASTASIS IN THE HEART MUSCLE.

E. LIBMAN, M.D.

In 1900 I published five cases of sarcoma of the small intestine and collected about fifty other cases from the literature.[1] Since that time we have had in the hospital ten further cases of this disease, in addition to which a small number of cases has also been reported by various other observers, most of which are collected in the valuable monograph of Lecene, published during the past year.

1 *American Journal Medical Sciences*, Vol. CXX, 1900, p. 309. *Mittheilungen aus den Grenzgebieten*, Bd. 7; Heft 4 und 5.

In none of the cases which I have seen or which I collected from the literature was there any note of metastatic deposits in the heart, and this is the first time we have encountered such marked involvement of the heart in any case of new growth.

The patient, a male, 35 years of age, was admitted to Mt. Sinai Hospital on July 26th, 1904, and died August 19th. He was under the care of Dr. Gerster, to whom I am indebted for the use of the clinical history.

Family History: Presents no points of any importance.

Previous History: Four years ago he had an attack of pain in the hypogastrium lasting two days, which was relieved by morphine. He had no chill at this time and no pain in the loins nor along the course of the ureter. The pains did not radiate; the urine was clear. Three and one-half years ago he had a similar attack; two years ago he had a third attack lasting five weeks, for the last two weeks of which time he was taken care of in another hospital.

Present History: Since the last attack he was well until four months ago when the hypogastric pain returned and he developed pains in the left loin, sticking in character. These pains did not radiate downward. For the last two months he has pain at the end of the penis during urination; the stream is never interrupted and the urine is always clear. He has been getting up once every night to urinate for the past three weeks. Three weeks ago he began to vomit all his food; this lasted one week. He has not been jaundiced. He has been in bed since the vomiting began. He thinks he has lost fifteen pounds in the last year.

Examination on Admission: The external lymph-

nodes are not enlarged except those in the submaxillary region which are slightly increased in size.

Heart: The dullness extends from the right border of the sternum to the nipple line; the apex beat is not made out; the sounds are weak; the first apical sound is short with very little muscular quality.

Pulses are equal, regular, rapid, of poor force, markedly dicrotic. There is marked sclerosis.

The lungs present the signs of emphysema with slight bronchitis.

The liver is enlarged to 1½ fingers below the free border of the ribs.

Spleen not palpable.

Abdomen is held rigid, is generally tympanitic; there is marked tenderness just above Poupart's ligament on the left side.

Genitals show no changes.

There is no edema of the legs.

Rectal Examination: Prostate negative. Commencing about 1½ inches above the prostate and extending up beyond the reach of the finger is a mass feeling about as large as an orange, extending somewhat more to the left than to the right, movable, nodular. Pressure exerted upon this causes pain to be felt at end of the penis. The tumor bulges somewhat into the anterior wall of the rectum.

The patient was operated upon on July 30th by Dr. Gerster. On opening the abdomen the appendix was found adherent to a large mass filling the true pelvis. The mass itself was found to be a tumor involving the intestines which would not permit of removal.

From the subsequent history of the case we shall give a few data only.

August 2d: Vomiting frequent; temperature 100 to 101° F. No pain.

August 5th: Bowels moved; no vomiting since the fifth day after operation. Temperature 101.6° F.

August 6th: Flatness over the right lower lobe; breath sounds distant and blowing; crepitant râles.

August 8th: A fecal fistula has developed.

On the 13th large lymph-nodes were felt in the left groin. There developed at the base of the left lung dulness and a few mucous râles. The patient died on the 19th of August.

The urine was usually of specific gravity of 1020 to 1030, contained a trace of albumin, a few red blood cells, pus cells, mucus and many bacteria.

The autopsy examination was restricted to an examination through the wound.

Abstract of the autopsy report:

Thymus: No traces found.

Esophagus: Moderately dilated.

Lungs: In the left pleura there is a large amount of fluid; in both lungs there are hemorrhagic infarcts and areas of new growth. The bronchial nodes are enlarged, pigmented and infiltrated. The posterior mediastinal nodes are also infiltrated.

Heart: Weight 510 g. On the surface of the right ventricle and auricle are several rounded hemispherical prominences, white in color, 2 to 3 cm. in diameter. All the chambers are dilated. The foramen ovale is slightly open. The wall of the right ventricle is thickened. The color is grayish red, particularly near the septum. There are a few small mural thrombi. The tricuspid valve is thickened. Left ventricle: The cavity is dilated; the wall is 2 cm. thick, is grey in color with a slight admixture

of red; it is rather friable. There is evidently a very marked diffuse infiltration by new growth. The mitral valve is thickened; behind one of the papillary muscles is an adherent decolorized thrombus.

The aorta and coronary arteries show fatty changes.

Spleen: Enlarged; marked perisplenitis; a few anemic infarcts; congestion.

Liver: Weight 1⅜ kilos. On section congested, cloudy, fatty; a few small areas of diffuse growth in the right lobe near the free border. Gall-bladder: Wall ½ cm. thick; diffusely infiltrated by new growth.

Kidneys: Several anemic infarcts in each kidney, also areas of new growth which shade gradually into kidney tissue proper; a few of these are elevated; pelvis dilated; ureters negative.

Pancreas and adrenals negative.

Bladder: There is infiltration by new growth of the posterior wall up to the mucosa; there are several blackish areas on the posterior wall; the vessels of the trigone are injected.

Stomach: Dilated ; wall acutely inflamed.

Intestine: In the duodenum there is infiltration of the wall, white in color for a length of 10 cm. The intestine is dilated at this point. Below this infiltration, between it and the main tumor, there are several places at which the intestine is infiltrated for short distances and is dilated. At a distance of 120 cm. from the beginning of the jejunum there is a very large dilatation of the intestine due to infiltration of its wall by new growth. This dilatation fills the entire true pelvis. The greatest thickness of the wall of the dilated intestine is 1½ cm. On section the growth is white and soft. The inner surface is necrotic and ulcerated. It is adherent to the bladder which is infiltrated by contiguity, and is also adherent to

the sigmoid flexure and coils of small intestine. It lies above the prostate, not attached to it.

The mesenteric nodes are enormously enlarged; on section they are white in color with yellow areas. The cecum is dilated. The rest of the colon is collapsed.

Superior mesenteric artery: Just above the bifurcation there is an adherent partially decolorized thrombus which extends slightly into both divisions of the vessel, not entirely filling their lumina.

Microscopic Examination:

A large amount of material from the case was studied, but only a brief report will be given here. The primary tumor of the intestine proved to be a lympho-sarcoma. The lungs, heart, kidneys, spleen, mesenteric nodes, liver, gall-bladder and urinary bladder wall showed metastases. The pancreas and adrenals showed no metastases. In the wall of the left ventricle and in the septum there was found to be a very extensive infiltration by the new growth, the individual muscle fibres being crowded apart by the cells of the tumor. There was marked degeneration of muscle fibres. The spleen and liver showed marked pigmentation. In the lung there were chronic interstitial changes and small purulent foci. The kidneys and liver showed parenchymatous degeneration. In the intestine the growth was found to begin in the submucosa and to infiltrate between the muscle bundles.

There are several points of interest about this case. First, it is a very good example of the dilatation which often occurs with lympho-sarcoma of the intestine. In this case the dilatation was more marked than any other I have ever seen.

Second, as is quite common in these cases there was

no ascites and no enlargement of the external lymph-nodes.

Third, the growth in the pelvis and adhesion to the bladder is a characteristic of some of these tumors which deserves more attention from a clinical standpoint than has heretofore been given.

Fourth, the duration of the disease is the longest that we have seen in any case. Although from the standpoint of the findings in the case we would ordinarily have diagnosed a lympho-sarcoma of the small intestine, the long duration of the case led us to suspect a non-malignant neoplasm or tuberculosis of the intestine with adhesions to the bladder. Baltzar put the duration of the disease at from two weeks to 1¾ years and said that most cases died in nine months. Rutherford described one case with a duration of 2½ years. In a series of cases which we have collected, this last-mentioned case was the longest one.

Fifth, the involvement of the heart was the most striking feature of the case. Although a note was made on admission to the hospital that the first heart sound had very little muscular quality, there was no suspicion that the heart was involved by new growth.

Discussion.

Dr. Harlow Brooks said that he had studied six cases of lympho-sarcoma involving the stomach and small intestine. Three of these six cases presented metastases in the heart. Some years ago he had looked up the literature and found that by far the larger percentage of cases where cardiac metastases occurred were of this kind. There was evidently a tendency on the part of the heart to accept these metastases of lymph-sarcoma.

A NEW METHOD FOR STAINING THE CAPSULES OF BACTERIA.

LEO BUERGER, M.D.

Dr. Leo Buerger spoke on a new method for staining the capsules of bacteria, and presented photographs and microscopic specimens. He said that though there were already a number of methods in use, the results obtained by them were, for the most part, variable and unreliable. Some six months ago while at work with streptococci in feces from both normal and diseased intestinal tracts, he had frequently met with lanceolate streptococci which morphologically closely resembled the pneumococcus. A similar organism had also been isolated from the uterus and vagina of a case of post-operative infection. He had therefore tried to devise a staining procedure which would enable one to recognize the pneumococcus on culture media soon after its isolation from the body.

Before describing the method itself, Dr. Buerger said that he wished to call attention to the particular characteristics of the capsule of the pneumococcus and some other organisms as shown by the method. The pneumococcus instead of presenting a narrow, poorly staining, elliptical, diffuse area about the body, showed rather a deeply staining, sharply defined, capsular membrane separated from the body by a clear zone. This was not always the case, for under certain cultural conditions and by more intense staining one could obtain a color between the pneumococcus proper and its capsular membrane. Dr. Buerger did not intend to discuss the variations in the morphology of the pneumococcus capsule on the different culture media or on media after a number of transplantations, but wished to confine himself to the

presentation of the typical forms. For this purpose he passed around a number of microphotographs. For these he was indebted to Dr. F. S. Mandlebaum, Pathologist to Mt. Sinai Hospital. Photograph No. 1 demonstrated the typical capsule of the pneumococcus in pure culture, showing besides some chains enveloped by a capsule and some empty capsules. Their appearance was so characteristic that the differentiation between this and other organisms in mixed cultures was easy. Other photographs made from specimens taken from throat cultures containing the pneumococcus together with streptococci and staphylococci, showed clearly the utility of the staining procedure. In another specimen made from empyema pus some two weeks after its evacuation from the chest, he pointed out the persistence of the capsule, although it surrounded markedly degenerate forms, and long chains.

In working with other organisms differences in the configuration of their capsules had been found. Thus, the so-called "Streptococcus mucosus capsulatus" is surrounded by a larger mass of more mucoid material. The latter owing to its gelatinous character is very easily broken up.

As regards the bacilli generally grouped under the name "Bacillus mucosus capsulatus," one could note the resemblance of their capsules to those just described. The amount of mucoid material surrounding the organisms depended not only upon the strain, but also upon the medium upon which they were grown. A specimen of a very large form isolated from the stool of a suspected typhoid case, was demonstrated. The capsule of this bacillus when grown on ordinary agar would often measure many times the size of the organism itself. Smaller forms enveloped by more linear outlines were more often to be met with in the throats of normal individuals; how-

ever, he had found the type described in quite a number of cases.

The streptococci, Dr. Buerger thought, showed as a rule, no true capsule. After having examined many strains with his method, he could group them in the following manner: Some could be classed with the "mucosus" group; most of them were absolutely free from any envelope; and others presented a linear membrane situated a short distance from the coccus proper. These latter should rather be spoken of as having an outer membrane or envelope, than as possessing a true capsule. However, it was to be noted that when the pneumococcus was grown under unfavorable conditions, or after many transplantations, its morphology was at times indistinguishable from the type of streptococcus just described.

Other organisms had also been stained, and it was found that the staphylococcus, meningococcus, gonococcus, micrococcus catarrhalis, showed no envelope. The Bacillus anthracis did, however, possess a distinct sharp line on either side and separated from the body by a clear space.

The method depended upon two things: the first was the spreading of the culture in a medium which would prevent dissolution of the capsule, and the second was the fixation of the capsule.

The necessary solutions were: (1) Zenker's fluid, minus acetic acid, *i. e.*, Müller's fluid saturated with bichloride of mercury; (2) Beef, human or other blood serum diluted with an equal amount of normal salt solution, or pleural fluid; (3) Alcohol, 80 to 95 per cent.; (4) Tincture of iodine, U.S.P.; (5) Freshly prepared stain, anilin water gentian violet, made up as follows: Anilin oil, 10 parts, water up to a hundred parts, shake, filter and

add 5 c.c. saturated alcoholic solution of gentian violet; (6) Two per cent. watery salt solution.

The technique was briefly given as follows: The culture is thinly and carefully spread over a perfectly clean cover slip by means of a drop of diluted serum. Just as the edges begin to dry the fixing fluid is poured on, the cover gently warmed over the flame for about three seconds, rapidly washed in water, flushed once with alcohol, and then treated with iodine for one to ten minutes. The iodine is in turn thoroughly washed off with alcohol, and the specimen dried in the air. Staining for a couple of seconds and washing with salt solution complete the procedure. The specimen is mounted in salt solution and ringed with vaselin.

As for the medium in which it was found best to spread encapsulated organisms, Dr. Hiss had pointed out the utility of serum. Although Dr. Buerger had been in the habit of employing beef serum, good results were obtainable by the use of pleuritic fluid and especially the sterile supernatant fluid from old thin empyema pus. It was important to pour on the fixative before the specimen was wholly dry, for in that way a great deal of the excess of serum was gotten rid of and a clear field was obtained.

As regards the fixing solution, saturated bichloride of mercury in one-half per cent. saline had also been used, but the results with Zenker were perhaps a little better. Dr. Buerger made note of the fallacy entering into many of the older methods in which there was either no or improper fixation of the capsule. The use of watery dyes would then seriously alter or wholly destroy the capsule. The bichloride was gotten rid of by means of the iodine. Dr. Buerger had attempted to shorten the process by discarding this step, but had come to the con-

clusion that it was indispensable in the preparation of a perfect specimen.

Dr. Buerger next discussed the question of the stains that could be employed. Although the ordinary anilin water gentian violet used for the Gram procedure was here also applicable, it gave as a rule too intense a stain, often fusing capsule and coccus into one. This was avoided by the use of the weaker solution above mentioned. Loeffler's methylene blue, on the other hand, was a little too weak; but strong, 10 to 15 per cent., aqueous fuchsin solutions (made from saturated alcoholic solution) gave very good pictures. Pappenheim's methyl green-pyronin labored under the same disadvantage as the methylene blue. However, it could be employed, the coccus taking on the red and the capsule the green color. It was very important to note that instead of a single stain the Gram procedure could be applied. Thus after the iodine was washed off with alcohol and the specimen dried, the ordinary Gram method was done. The specimen was best counter-stained with 10 to 15 per cent. fuchsin which remained on the cover slip for a minute or longer, and was washed and mounted in water. In this way the body of the pneumococcus took on a deep violet color and the capsule a red tinge. The Friedländer bacillus on the other hand, had both a red body and a red capsule. The small diplococcoid forms of this bacillus when occurring alone or together with the pneumococcus in throat cultures or other mixtures were easily recognized and differentiated from the latter. In routine examinations of throat cultures made on the Loeffler medium by his method, Dr. Buerger had frequently noted the similarity of the small Friedländer bacillus and the pneumococcus. This had led to the observation that some such differential method was at times necessary. With

the combined procedures a diagnosis can be made at once.

Exudates and pus do not generally require the addition of serum. The very mucoid stringy purulent exudates and sputa, however, are best thoroughly mixed with serum before fixation and staining.

In conclusion Dr. Buerger called attention to the utility of the method, not only in the demonstration of the capsules of encapsulated bacteria in exudates and culture media, but also in bringing out clearly some points in the finer morphological structure which are not recognizable with the ordinary stains.

A CASE OF "SPLENOMEGALIE PRIMITIF" WITH INVOLVEMENT OF THE HÆMOPOIETIC ORGANS.

N. E. BRILL, M.D.

Dr. N. E. Brill spoke on a case of primitive splenomegaly with involvement of the hæmopoietic organs, reviewing the literature and giving the clinical history of his case.

Under the caption of primitive or primary splenomegaly, he said, there had been heaped together a large variety of non-malignant enlargements of the spleen. Osler in an attempt to establish order out of chaos had suggested that those cases of splenic hypertrophy with or without anæmia and associated with hæmorrhagic tendencies should be classed under the name of splenic anæmia. Osler considered that the enlargement of the liver in the group of cases to which Banti called attention was but a

terminal feature of the disease called splenic anæmia. Attention was first called to the group to which Dr. Brill's case belonged by Gaucher, Picou and Raymond, and Collier. More recently two cases had been described by Dr. Bovaird. This class of cases, called by Gaucher epithelioma of the spleen, should be taken from among the group of splenic anæmia, because its clinical history and pathology were distinct. The clinical history of Gaucher's type is so distinct that Dr. Brill had been able to state that his cases published about five years ago did not belong to the category of splenic anæmia, but belonged to this type. The subject of the present demonstration was one of these cases. The patient died in March, 1904, after having been under observation since 1885.

The heredity of this young man showed nothing of this type of disease. His history had been traced as far as the great-grandfather on the maternal side and to the grandparents on the paternal side. In none of these ancestors was there any suggestion of this disease. The disease has appeared only in one generation. The parents of the patient had six children. The oldest died at the age of three of marasmus. The second one is now a woman of 45 years and is in good health. The third child was one of the patients described in 1901. The fourth was the subject of this demonstration. The fifth had shown in the past two years evidences of the disease. The sixth had died at the age of nine, having shown from his third year a splenic enlargement, from exhaustion after tremendous increase in the size of the spleen and liver. Thus four out of six children have had the disease. This patient had the usual infectious diseases of childhood, measles, scarlet fever, etc. He never had malaria nor tuberculosis nor syphilis. From his third to

his ninth year he was not very strong but afterwards grew into a strong chap, and had no complaints whatever except occasional bronchitis. At the age of fourteen he began to notice that he perspired rather freely; otherwise he was in perfect health. In 1888, when his sister was married, there having been no occasion for a previous examination, she herself called attention to a mass on the left side of her abdomen. On examination a tremendous enlargement of the spleen was made out. The brother was also examined and there was at that time no enlargement whatever. The next year the spleen could be felt in the patient who was under consideration, the upper portion extending to the seventh rib. His disease thus manifested itself first in 1889, fifteen years before his death. At this time he began to have attacks of epistaxis. These were the only manifestations of hæmorrhage for some years. They would occur four to six times a year, were rather profuse and lasted for a short time. During this period there were no signs of anæmia. About four years afterwards, the enlargement of the spleen going on very slowly, a beginning thickening of the conjunctiva on the nasal side appeared. This was yellow white in color, and was much firmer and denser than a pinguicula. This symptom was also noticed in the sister and has since appeared in the other sister.

During this time the blood showed no changes. There was a normal number of red cells, varying from 4,500,000 to 5,600,000. There was no increase in the white cells, and no disturbance of the normal relations of the leucocytes. The spleen continued to increase in size and nine years afterwards the liver began to show enlargement. In 1900, the patient had an attack of acute colitis, preceded by a chill and followed by a temperature of 106.5. This was accompanied by numerous watery

movements and after two days by some blood and mucus. From this time the disease progressed very rapidly. During convalescence from this attack, a number of hæmorrhagic furuncles appeared on the body, the largest on the extremities. When these discharged their contents they left a permanent pigmented spot. A change in the appearance of the parts of the body which were exposed to the light and air also manifested itself. The skin of the face and hands became yellowish brown in color. There was no icterus at any time. There was no further pigmentation except that left from the furuncles. In 1902, the first sign of pain manifested itself in the region of the spleen. The spleen extended down to the crest of the ilium by this time. Over the bridge of the nose was a persistent erythema which had made its appearance when the patient was fourteen years old and which lasted until his death. In 1902, for the first time, pain was noticed at the extremities of the tibia and femur. Up to this time there had been no bone pain. In June, 1902, the patient contracted malaria and the plasmodia were found in his blood. After this attack the first tendency to hæmorrhage manifested itself. There now appeared permanent petechiae scattered over the hands, legs and trunk. During 1903, he had a fairly favorable year, notwithstanding the tremendous increase in the size of the spleen and liver. He complained of no discomfort whatever. After 1895 the first sign of anæmia appeared. The hæmoglobin was about 80. From 1895 it gradually diminished, losing perhaps 1 or 2 points a year until 1900, when after the attack of colitis it suddenly fell to 65. From this time on to the end of his life on March 30, 1904, the hæmoglobin diminished until it reached 45. In March he complained of dyspnœa. On examination the signs of pericarditis were manifest.

The area of cardiac dulness increased rapidly, establishing a diagnosis of pericardial effusion, which Dr. Brill assumed to be hæmorrhagic. Death occurred on March 31, 1904. The autopsy was made by Dr. E. Libman at midnight. Dr. Libman was asked to present the results of the autopsy and Dr. F. S. Mandlebaum the pathological histology of the affected organs.

Dr. E. Libman showed the liver and spleen from the case described by Dr. Brill, and also presented those from the second case which Dr. Brill had mentioned. The spleen in the first case weighed eleven pounds, the liver ten pounds. In the second case, the spleen weighed fourteen pounds, the liver over eight pounds. The organs in both cases presented the same findings, except that atheroma of the splenic vein was absent in the first case.

The spleen was very firm. It was chocolate in color with lighter grayish-red areas. The pulp was swollen. There were numerous infarcts present, some recent, some old. Many of these were in the periphery of the organ. There was no evidence of marked fibrosis to be seen, except where the pulp was less swollen. The measurements were 40 x 20 x 14 cm.

The liver measured 35 cm. in width, either lobe, 27 cm.; the organ was 13 cm. thick. There was marked old and recent perihepatitis. On section, the organ was chocolate colored, of firm consistency; it appeared rather granular. Throughout the viscus there were irregular white markings varying in diameter from 0.5 to 2 or 3 cm. They did not seem to bear any definite relation to the lobular markings.

The pericardium was distended by two liters of very bloody fluid.

The lymph nodes in the thorax and abdomen were enlarged. The largest were about the size of a large bean. They were fairly soft, and were of a peculiar ochre color, some showing hæmorrhagic areas.

The bone marrow in the first case was quite firm, dark red in color. In the second case it was partly red and partly ochre-colored.

Dr. F. S. Mandlebaum described briefly the histological picture of the case. The characteristic feature of the disease was a very marked endothelial hyperplasia. This was found in the spleen, liver, lymph nodes, and bone marrow. The picture found in the case followed very closely, with a few exceptions, the cases described by the French writers and Dr. Bovaird, as mentioned previously by Dr. Brill.

In the spleen the endothelial hyperplasia occurred in round or oval alveolar spaces. These spaces were the pulp spaces or venous sinuses, and were lined with endothelium which was normal in some areas and slightly swollen in others. The endothelial cells as a rule filled these spaces completely, though some of the alveoli contained a few normal pulp cells, while others were more or less filled with red blood cells. The endothelial cells varied in size and shape, depending on the number in an alveolus. For the most part they were round, oval, or polygonal. The boundaries of the cells were fairly well distinguished, as a rule, but in some places the cells were fused together, and the cell outlines then were not clearly seen. The cytoplasm of the cells were slightly granular,

or streaked with fine wavy lines, arranged in a more or less concentric fashion, giving a wrinkled appearance to the cells. The nuclei were relatively of small size. The cell body measured anywhere from 17 by 21 micra to 34 by 47 micra, while the nuclei varied from 5 to 8 micra in size. In some of the cells several nuclei were found, as many as six or more being present in some cases. These were not arranged in any particular form, but were scattered throughout the cell. No forms presenting the usual appearance of giant cells were found.

In other parts of the spleen dense bands of connective tissue were seen, also anæmic infarcts containing small areas of necrosis. The Malpighian bodies were for the most part absent, though occasionally one could be seen showing a slight thickening of the walls of the central vessel. A large amount of pigment was scattered throughout the sections. This was seen along the connective tissue bands and sometimes in the alveolar spaces.

The sections of the liver presented the ordinary picture of a diffuse cirrhosis. There was a marked increase in the interlobular connective tissue without apparent effect on the liver cells. The bile ducts were not affected. The endothelial cells described in the spleen were also found in the liver. For the most part they were seen in the connective tissue spaces, but were not so clearly defined as in the spleen. They were also found in the lobules proper, either near the periphery or in the capillaries extending towards the center of the lobule. The cells were somewhat smaller in size than those in the spleen, but otherwise were identical in their general appearance and staining reaction. Pigment was also found distributed throughout the connective tissue of the organ.

The sections from the lymph nodes were so changed that they appeared simply as one mass of endothelial cells with a remnant of a follicle remaining here and there. With the low power one could see large masses of pigment in the lymph sinuses and also following the reticulum of the node. The endothelial cells had the same general appearance as in the spleen and liver, but their outlines were more definitely preserved. Some very large forms were seen in these sections. The pigment in the nodes as well as that found in the spleen and liver gave the usual reaction for iron.

In the bone marrow these endothelial cells were also found, and Dr. Mandlebaum thought this to be the first case of splenomegaly in which their presence in the marrow had been noted. They were found attached to the walls of the capillaries and to the reticulum of the bone marrow. In several places these cells were seen in the lumen of the capillaries. They had the same features as in the other organs.

As far as the deductions to be drawn from these sections were concerned, Dr. Mandlebaum did not feel justified at the present moment in coming to any conclusions as to the consecutive involvement of the various organs, or whether the process occurred simultaneously.

A modification of Mallory's connective tissue stain devised by Dr. Buerger had been used in staining some of the sections. This brought out the markings in the cytoplasm very distinctly, and also differentiated the nuclei better than most of the other stains employed.

Discussion.

Dr. David Bovaird said that these cases were of very great interest from two standpoints. First, the

clinical; he thought they proved that there was a definite group characterized by this primary enlargement of the spleen which should be separated from the other groups of cases. Shortly after the publication of his own paper, Dr. Brill had called his attention to this series of cases in one family. Dr. Brill had said at that time that the cases reproduced the clinical picture of the cases described by Dr. Bovaird. In other words, Dr. Brill had made a clinical diagnosis of a condition exactly that found in these children, and the autopsy findings proved that this diagnosis was correct. From the pathological standpoint the condition was of interest in the interpretation put upon the findings. Dr. Bovaird thought that the question really lay between whether this condition should be called a hyperplasia or whether it should be classed as a tumor and called endothelioma. At a recent meeting of the American Association of Physicians the opinion had been expressed that these conditions belonged in the class of endothelioma. Whether this condition was a tumor or an hyperplasia was one of those questions which only pathologists could discuss to advantage. It seemed that even among pathologists the conception of what constitutes a tumor was not clear enough to enable one to determine the exact position of changes such as these. The clinical history of these cases seemed to point rather clearly to some influence running in a family rather than to a new growth. It would of course take many other observations and much further study to determine this point.

Dr. Libman said that the absence of disease of the splenic vessels in the first case was noteworthy. In the condition described by Banti it was usually present. The microscopic changes in the bone marrow were of great interest. There were present many nucleated erythro-

cytes, lymphocytes and mononuclear cells, neutrophilic and eosinophilic myelocytes and giant cells in abundance. The occurrence of such changes in the marrow with the absence of abnormal cells in the blood was a remarkable finding. Dr. Libman thought that the cases showing the same lesions as this one—those of Gaucher, Picou and Raymond, Collier and Bovaird—should be grouped separately from other forms of splenomegaly, and for the time being be called "splenomegaly of the Gaucher type."

Dr. Osler had seen the microscopic sections and had noted the resemblance to Gaucher's findings.

RECENT OBSERVATIONS ON PARASITES OF THE MOSQUITO.

W. N. BERKELEY, M.D.

Dr. W. N. Berkeley reported some recent observations on parasites of the mosquito. The first specimen was a minute nematode worm, called by Mr. Henry L. Viereck, of the Connecticut Agricultural Experiment Station, *Agamomermis*. The speaker was indebted to Mr. Viereck for the beautiful preparations shown, illustrating the egg, larva, pupa, and imago of the mosquito concerned (*Grabhamia sollicitans*, formerly *Culex sollicitans*), and the parasites. Mr. Viereck wrote that he had found *Agamomernis* only in the female mosquito, usually one only, but sometimes as many as five, in one body. In parasitized mosquitoes, eggs had been found only in one or two individuals out of hundreds examined. The same or certainly a very closely allied parasite had been reported as found in a dish of water in which pupæ of Anopheles

had been reared (H. P. Johnson, *Bulletin of New Jersey Agricultural Experiment Station*, November 29, 1902). A very similar worm was described as having been noticed many years ago by Leuckhart (Howard, quoting from Stiles, Mosquitoes, New York, 1901). The worm was easily recognized as a nematode under the lower power of the microscope. The speaker had never had the good luck to find a specimen in his own work.

The second specimen was a red mite, smaller than a small pinhead (1.5 mm.) which he had discovered on two occasions attached once to the thorax, once to the abdomen, of one of the common Culex species (not worked out carefully, probably *C. impiger* or *triseriatus*) in the City of New York. The mite appeared to do little or no damage to its host. A specimen had been kindly identified for him by Mr. Nathan Banks of the United States Department of Agriculture—through Dr. Howard's good offices—as the larva of a *Hydrachnid* belonging to the genus *Eylais*. Dr. Howard had written as follows: "The adults of these mites are parasitic upon the water-bugs or water-striders, usually of the family *Nepidae*. Their presence upon the mosquito is probably accidental, they being probably in no sense specific parasites of the *Culicidae*."

Dr. Berkeley feared that both specimens were mainly of academic interest, but thought that in view of the vast hygienic and economic importance of mosquitoes, their parasitology was worthy of systematic study.

STUDIES ON THE ETIOLOGY OF MEASLES.

CYRUS W. FIELD, M.D.

Dr. Cyrus W. Field said that these studies of measles were begun in connection with some observations of Dr.

Duval's during the summer. Dr. Duval devised a method of obtaining blister fluid in scarlet fever cases to see if he could obtain the parasites described by Dr. Mallory. He used the Wright stain and therefore in a great majority of the slides there was absolutely no sign of chromatin stain. These bodies stain a pale blue with a little darker blue granules throughout. After seeing these specimens Dr. Field had made some blisters on scarlet fever patients and also on measles and diphtheria patients. In scarlet fever, out of 18 cases, these bodies were found in 14. Thirteen cases of measles were studied. In the first six days of the rash the bodies were found in 8 cases. After the fifth day to the tenth day they were not found. They were not found in three cases of diphtheria antitoxin rash, in erysipelas, in hereditary syphilis, in an urticaria rash, in normal skin, or in irritated normal skin. The bodies found were very definite and characteristic. The ones considered as being of interest were those which stained blue with a chromatin staining substance in them in the form of granules or solid dots. The specimens were all stained with Hastings' or the Giemsa stain, the latter being much stronger. Some hanging drops and spreads had been made which showed some very suspicious occurrances. For instance, there were a number of leucocytes in the different fields which gave off pseudopodia which would work off very rapidly and in a short time assume a definite round appearance with two, three, or five granules dancing rapidly and giving a much more striking appearance than many malarial parasites. They were very numerous in freshly drawn serum, and when stained showed that these small granules were chromatin like in their reactions.

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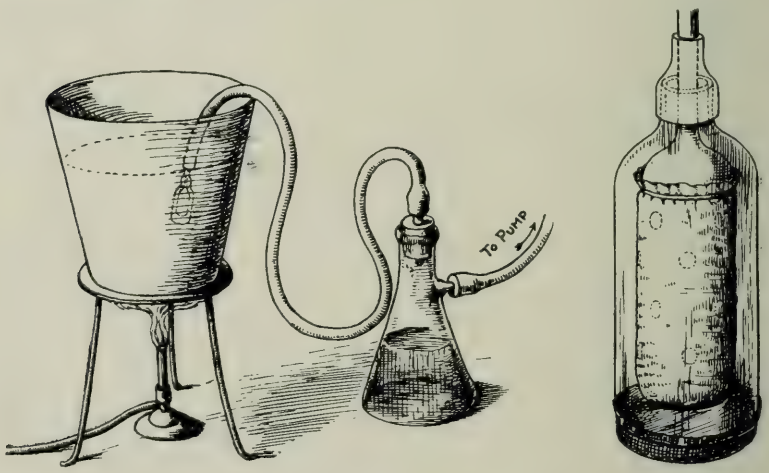
DR. OTTO H. SCHULTZE, *President.*

A SIMPLE METHOD OF FILTERING AGAR.

J. FINLEY BELL, M.D.

Dr. J. Finley Bell demonstrated a method for the rapid filtration of large or small quantities of agar. The apparatus was exceedingly simple, consisting of a cylindrical glass bulb with perforations over which was slipped a Schleicher and Schüll thimble of filter paper of the type used in the Soxhlet fat extraction apparatus. The whole was covered with a cylindrical glass protector slightly larger than the bulb. The open end of the protector was covered with several layers of gauze and some absorbent cotton to strain out the coarse particles. The other end was attached to a piece of heavy rubber tubing and connected to a filter flask. A vacuum was produced in this flask by means of an ordinary Bunsen filter pump.

If the filtering apparatus is then moistened with hot water and dropped into the vessel containing the hot agar, and the pump started, filtration will take place rapidly, about a liter being filtered in three or four minutes. The advantage of the apparatus consists in the fact that no cooling takes place until the agar has passed through the filter, whereas with the ordinary method of filtering agar it is difficult to prevent the agar from thickening or becoming solid in the filter. The reaction can finally be very easily adjusted in the hot agar after filtering. The accompanying plate will render the construction of the apparatus clear.



CYST OF THE KIDNEY.

R. WEIL, M.D.

Dr. Richard Weil presented a specimen of a cyst of the kidney. This was originally described as "malignant cyst of the kidney" by Struebing. It is actually an ex-

ample of a rare, yet important type of the adrenal tumors of the kidney.

The specimen was removed at operation by Dr. Kammerer of the German Hospital. The patient had previously been exhibited at many societies, and, although the cystoscopic findings pointed indubitably to the presence of a renal disease, the exact pathological condition was never diagnosed. The same failure marked all the other cases recorded in the literature, in which the clinical diagnosis ranged from echinococcus cyst of the kidney to cyst of the ovary. Yet, as Struebing says, there are certain fairly characteristic symptoms which should really serve to differentiate it. Pathologically, it has been exactly duplicated by seven other undoubted cases in the literature; yet there are numerous other descriptions of renal cystic tumors, both in the older and in the more recent literature, which most probably belong to the same category, although the genesis of the cyst from an adrenal tumor was not identified. And that it could easily escape such identification was illustrated by Dr. Weil from the specimen in hand.

It presented a large monolocular cyst, partially subdivided by fibrous septa. Roughly, it had the shape of the kidney. The wall was formed by a dense white tissue of varying thickness. The content was a thick, dark brown, grumous fluid, which contained leucocytes, red cells, epithelia, blood pigment, and cholesterin.

Closer examination revealed the presence of two inconspicuous strands of a different tissue, one of which lay in the capsule, the other in one of the septa. Both of these strands were inclosed in a distinct fibrous capsule. Their tissue was firm; their color, whitish yellow. On microscopic examination they were found to be adrenal epitheliomata, presenting the characters of this tis-

sue as it appears after it has undergone malignant degeneration. In some places it was with difficulty distinguishable from cancer; in others, it was distinctly of the structure of the adrenals. The wall of the cyst itself presented the remains of the kidney—glomeruli and tubules imbedded in a dense mass of connective tissue.

Certain details of the clinical history were adduced by Dr. Weil as of interest, not only for themselves, but as throwing light on the growth and development of the tumor.

The patient was fifty-six years of age. Up to the age of sixteen he was perfectly healthy. At that time he passed bloody urine almost constantly for two weeks. He had no pain and no other evidence of disease. From that time until 1899, a period of thirty-five years, he was in every respect perfectly well. In 1899, at the age of fifty-one, he again had an attack of hematuria which persisted for several weeks, and left him quite weak and anaemic. Since then he had had recurrences every year.

He entered the German Hospital in June, 1904. He was markedly anaemic, blood count less than 3,000,000; hemoglobin about 40 per cent.; and he looked cachetic. His abdomen was markedly distended by the cystic fluctuating tumor. His urine contained blood, which cystoscopic examination showed to flow from the left ureter. Puncture of the cyst yielded a bloody fluid. Since the removal of the tumor he has steadily gained flesh and strength and the anaemia has disappeared.

The remarkable facts are, the existence of the tumor for a period of forty years; its cystic character; its non-malignancy, except as a cause of anaemia.

The other cases recorded in the literature were exactly similar to this in the pathological findings. Clinically, they differed chiefly in the presence or absence of

blood in the urine, depending upon the communication of the tumor sac with the pelvis of the kidney. In those cases in which there was no hematuria, puncture of the kidney cyst always yielded a fluid of the same character, containing blood pigment and cholesterin.

Discussion.

Dr. F. C. Wood said that this very interesting cyst of the kidney reminded him of a case he had seen six or seven years ago, which corresponded to Dr. Weil's description of the case of hemorrhage into a suprarenal tumor, with the formation of a large cyst. The cyst he had seen was about the size of a child's head, lined on its inner surface with shaggy clots and a few projections containing suprarenal tissue but no smooth walls. It was, therefore, of a slightly different type, probably a simple hemorrhagic cyst with softening of tumor tissue.

Dr. Weil said they were clinically of some importance. All described cases had been given the wrong diagnosis. Many had been considered echinococcus cases, others as cases of cyst of the ovary. This tumor simply produced pressure symptoms; the content was invariably bloody with cholesterin from breaking down of the cells. That gave one a hold on the diagnosis.

A CASE OF TRICUSPID AND MITRAL STENOSIS.

BOND STOW, M.D.

Dr. Bond Stow presented a specimen showing tricuspid and mitral stenosis, together with the lungs showing very extensive hemorrhagic infarcts. He said that

since one seldom saw a marked case of tricuspid stenosis with such extensive sequelæ throughout the lungs, he took pleasure in presenting to the Society these specimens. He afforded a view of the heart from the right auricle looking towards the right ventricle, demonstrating the tricuspid valve with its three cusps joined together for a distance of 2.5 cm. from the base of the valve to its closing margin. The opening through the tricuspid valve measured about 1.5 cm. in diameter. The right ventricle measured at the apex 4 mm. and at the base of the tricuspid valve, 1.5 cm. The right auricle was very extensively dilated and its walls averaged about 3 mm. On the right auricular appendix were several thrombi, one being about the size of a hickory nut. Looking from the left ventricle through the mitral valve into the left auricle, Dr. Stow demonstrated a mitral stenosis that scarcely admitted the tip of the smallest finger. Along the base of the anterior flap of this valve was a firm, stony hard, extensive, calcareous deposit in the form of a ridge fully 2.5 to 3 cm. in length. The left ventricle did not appear to be either hypertrophied nor dilated. The left auricle was much dilated and its walls measured about 3 mm. in thickness. The left auricular appendix was filled with thrombi.

At the apex of the left lung was an old infarct which had partially undergone necrosis and was more or less cavernous and filled with a foul smelling detritus matter. There had been firm pleuritic adhesions over this infarct. In the right lung, along the anterior borders of its upper lobe, was a large recently formed hemorrhagic infarct. The entire middle lobe was one hemorrhagic infarct. About four-fifths of the lower lobe likewise was one large, apparently recently formed infarct. There had been complete thrombosis of the external jugular on the left

side and also a canalized thrombus was found in the superior vena cava. The right kidney also showed innumerable hemorrhagic infarcts. All of these the speaker believed were due, not to embolism, but to autochthonous thrombosis, owing to the favorable circulatory conditions brought about by the tricuspid stenosis, all thrombi found in the blood vessels being firmly adherent to their walls. The liver and other internal organs showed the ordinary appearances of cyanotic induration.

Dr. Stow also demonstrated a specimen from his recent post-mortem work, showing the anomaly of four cusps to the pulmonary valve, which condition had had no effect upon the welfare of the patient and had proven a mere post-mortem disclosure.

EMBOLISM OF THE LEFT MIDDLE CEREBRAL ARTERY.

M. G. SCHLAPP, M.D.

Dr. M. G. Schlapp presented a specimen showing embolism with following thrombosis of the left middle cerebral artery after ligation of the common carotid. Dr. Schlapp said that he had Dr. James Johnston to thank for this specimen.

The case in question was a man, forty-eight years old. Three years previous to his admission into the hospital, an epithelioma began to develop on the lower lip. Some time after, the submaxillary glands and the surrounding areolar tissue became involved, so that when he was seen by the surgeon the lower jaw and submaxillary tissue were diffusely invaded, so much so that the ligation

of the common carotid was advised in order to check the growth. This was done. The carotid was ligated about two inches below the point where the common divides into the external and internal carotids. Thirty-six hours after the ligation the man suddenly became paralyzed on the right side (hemiplegia) and lost consciousness. Twenty-two hours after the onset of the hemiplegia, exitus letalis took place. At the autopsy the following condition was found. The common carotid was opened below and above the ligature to the point where it divides into the external and internal carotids. It was found that a thrombus completely filled the common carotid from the ligature up to the division of the vessels and that it partly extended into the internal carotid for about 2 cm. Furthermore, it was seen that the thrombus did not completely fill up the internal carotid, but that an open space was left extending from the external carotid (which contained no thrombus) into the internal carotid. As was stated above, the thrombus extended about 2 cm. into the internal carotid from the common, and above this the internal carotid was clear up to the point where it passed into the middle cerebral artery. The latter was completely thrombosed. The upper end of the thrombus which protruded into the internal carotid, was torn and irregular. The smooth dome-shaped point which is invariably found at the end of a thrombus in a large blood vessel was missing.

The explanation of the case was as follows:—After the common carotid was ligated, a thrombus formed above the ligature, growing to the point where the common divides into the external and internal carotids. Here it grew some distance, but not into the external carotid because this vessel was filled with fresh flowing blood coming through collaterals from the opposite external

carotid and flowing back into the internal carotid. This stream of blood from the external carotid into the internal carotid washed off the end of the thrombus extending into the internal from the common carotid, by which it was washed up into the middle cerebral artery closing up this artery and causing sudden hemiplegia and unconsciousness and later death. Encephalomalacia was distinctly seen in the area supplied by the middle cerebral artery.

Dr. Schlapp wished to show this case to point out the danger in ligating the common carotid without ligating at the same time the external carotid.

NOTES ON VARIATIONS IN VIRULENCE AND ON SPONTANEOUS AGGLUTINATION.

EDNA STEINHARDT, M.D.

Dr. Steinhardt read a paper on the above subject.

The variations in virulence studied were those obtained by passing bacteria through immune and through bactericidal sera, following the methods of Ainley-Walker (*Brit. Med. Journal*, 1902, and *Centralbl. für Bakt.*, 1 Abt. Org. Bd. 33, 1903). The effect on the toxin production was also noted by growing the organisms in antitoxin.

The experiments fell under three heads:

1. Effect on the toxin production of diphtheria bacilli by growth in antitoxin.
2. Effect upon the virulence of dysentery bacilli by growth in inactive horse serum, normal and immune.

3. Effect upon typhoid bacilli of repeated passages through bactericidal horse serum.

1. In the first experiments two varieties of diphtheria bacilli were grown for fifteen generations in diphtheria antitoxin, controls being kept in broth. They were then tested for toxin production, and in both cases the serum-grown strains produced less toxin than the controls.

From these experiments it can only be stated that there was no specific increase in toxin production caused by growth in antitoxin. The antitoxin in which the bacilli had been grown lost markedly in protective value due to direct neutralization by the toxin produced.

2. Dysentery bacilli of the mannit-fermenting type were grown in inactive, immune and normal horse sera. Two dilutions of each were used, strong and weak, as different results had been obtained by other workers with different dilutions, thus suggesting the possibility of over stimulation of the bacilli, causing decrease instead of increase in virulence.

The cultures were grown for five weeks and then tested. It was found that all the strains had lost in virulence except that grown in normal weak serum; this had retained its virulence. To control these results a second series were grown for twelve generations in immune serum, strong and weak dilutions; that grown in strong lost markedly in virulence, about one half; while that in weak retained its virulence.

3. Typhoid bacilli were passed through lytic serum forty-three times and then tested. They had no increased serum-resistance in vitro, but had doubled in virulence. To control these results, from the original stock a culture was again passed through lytic serum ten times. In this instance there was greatly increased serum-resistance in vitro and the virulence was sevenfold. The serum-resist-

ance in vitro was found by reactivating experiments to be due to increased resistance to immune body and not to resistance to complement, as found by Erich Cohn (*Zeit. für Hyg.*, Bd. XIV). Two other series were done from the same stock culture, and in exactly the same manner; one series five times, and one ten. In neither instance was there increased serum-resistance in vitro; the virulence was not tested.

Thus in four parallel experiments, serum-resistance was obtained only once, and virulence was increased in much greater degree in one case than in the other.

From the results obtained in these experiments, the following conclusions may be drawn:

1. If there be a specific reaction between the receptors of the bacillus and the amboceptors of the serum, a certain proportion would be necessary to cause the overproduction of the bacillary receptors, as too great a number of amboceptors might overwhelm the bacillus, thus causing loss of virulence. This may be the reason for the irregular results obtained, or these results may be due to a non-specific action of the serum which, up to the present, is not understood.

2. In four series of passages of typhoid bacilli through bactericidal horse serum, under the same conditions, uniform results were not obtained. A definite reaction cannot in any given case be predicted. In some instances the bacilli surviving after the action of the lytic serum are the more resistant ones. The negative results may be due to the fact that in some instances the bacilli may possibly be protected from the serum mechanically, by being in the center of an agglutinated mass, or it may be that the bacillus must be in a certain condition to react,

as some animals of the same species are more easily immunized than others.

Spontaneous agglutination has been noted a number of times in serum-grown organisms by other workers. In these experiments, it occurred in the series passed forty-three times through bactericidal serum, after the twentieth passage. Over twenty generations on agar retained this characteristic.

Returning to the stock typhoid culture, which grew occasionally with a slight pellicle, it was found possible by repeated plating to separate from it two variations, one giving a uniform cloudiness, the other agglutinating spontaneously in broth. This spontaneous agglutination is not an agglutination in the specific sense of the term, as from an agglutinating typhoid serum the agglutinators absorbed the same number of specific agglutinins as the non-agglutinating stock culture. The spontaneous agglutination continued through a number of generations, but by sub-culturing from a shaken broth culture, in a very few generations, this variation grew in broth with the original uniform cloudiness.

Further work was done on two unidentified specimens isolated by Dr. Goodwin, one from water, the other from the intestinal tract. Both these organisms agglutinated spontaneously when transferred from agar into broth. In broth they produced cloudiness and formed a pellicle. Here it was also possible to separate variations, one giving only pellicle, the other, clouded broth with very slight pellicle production.

These results suggest that in cultures in which spontaneous agglutination occurs two variations exist in the original culture—one tending to grow in clumps, the other diffusely,—and that under certain conditions the one type predominates, under other conditions the other type.

Discussion.

Dr. C. W. Field said that the point of spontaneous agglutination was very interesting, and might throw light on the meaning of the reaction of agglutination. Dr. Steinhardt had said in her paper that it was not specific agglutination, but under the microscope it was practically impossible, so far as he had seen her preparations, to tell the difference between these and organisms agglutinated by specific serum. They had the same appearance, the same large clumps, and clearness of the field, that are found in sera of high specificity, and they were intensely interesting from that standpoint. The presence or absence of agglutination might not be an entirely specific reaction of the serum on the organism itself, but might be due to variations in the ability of the organisms to absorb certain constituents from the media.

Dr. E. Libman said that from the few observations he had made regarding spontaneous agglutination he considered also that it could at times be found apart from any action of serum. He had experimented with a paracolon culture—Dr. Cushing's bacillus. In that culture, after it had been grown for several months in ordinary bouillon, spontaneous agglutination occurred regularly after each transplantation. Jaeger in his agglutination work could not use some cultures because of spontaneous agglutination in salt solution. Dr. Libman thought pellicle formation depended to a great extent on the presence or absence of a carbohydrate content from which the organisms could derive oxygen. An organism which would grow with marked pellicle formation without sugar would often grow without or with a slight one if a carbohydrate were present which it could use. From the experience of the speaker he would not feel justified in

saying that a distinct relationship existed between pellicle formation and spontaneous agglutination.

Dr. Steinhardt said that she thought that if Dr. Libman had shaken his broth culture of the paracolon bacillus, had then plated it, and had picked out the translucent thin colonies, repeating this several times, he would have gotten a non-clumping culture again from his spontaneous agglutinator. She had done so and had obtained a non-clumping culture. As to culture media, the same broth was used in both cases. By separation one variation would produce pellicle and the other not. This was not due to a difference in sugar content because exactly the same medium was used.

Dr. Libman said he had plated the paracolon bacillus out a great many times, had tested many colonies, and had got spontaneous agglutination in bouillon subcultures regularly. He was very anxious to get it to grow diffusely but could not succeed.

Dr. Field said it was not due to serum present. In one case it was not due to serum especially as the serum did not come in contact with the organism.

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